EVIDENCE GENERATION FOR EFFECTIVE CHOLERA OUTBREAK INTERVENTIONS IN KATHMANDU VALLEY, NEPAL

By

Mellisa Roskosky, MSPH

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ABSTRACT

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Objectives

Cholera is endemic in 69 countries and is responsible for at least 95,000 annual deaths, over 900 of which are in Nepal. Studies demonstrate a higher risk of cholera in the vicinity of a case. A Comprehensive Targeted Intervention (CTI) was designed and deployed adjacent to cholera cases in the Kathmandu Valley with the intent to reduce rates among neighbors. This dissertation aims to determine if an immediate, integrated response is possible in the Kathmandu Valley while simultaneously generating data to support evidence-based interventions to control cholera in the country.

Methods

Cholera cases were reported from 15 sentinel site hospitals. A single case initiated the CTI. Bacterial culture was used for confirmation. The strategy included case investigation, water testing, WASH intervention, and health education. Stool samples were collected on filter paper for testing genetic relatedness. Case household location data was analyzed to determine if a reactive, ring vaccination strategy would have been useful in preventing cholera transmission during the outbreak.

Results

Between June 1 and December 30, 2016, 169 cases of *V. cholerae* were confirmed by bacterial culture. On average, the CTI Rapid Response Team (RRT) was able to visit the

household 2 days after the culture result was received. PCR testing confirmed an additional 24 cases, and MLVA revealed that all samples were members of a single clonal complex. GPS data was available for 69 households and significant clustering of cases was seen over space and time. Approximately 85% of cases within a cluster occurred more than seven days after the index case.

Conclusion

RRTs were able to visit case households within 48 hours of confirmation and were successful in raising awareness among key stakeholders. The minimal genetic diversity in the clinical samples combined with the shape of the epidemic curve indicated a clonal outbreak consistent with a common source followed by secondary fecal-oral spread. Thus, it seems unlikely that there were multiple introductions of *V. cholerae* into the Kathmandu Valley in 2016. Clustering suggests an opportunity to prevent cholera cases though ring vaccination. This analysis has provided useful evidence for implementing future cholera outbreak interventions in Nepal.

COMMITTEE OF THESIS READERS

Thesis Readers:

Kenrad Nelson, MD; Committee Chair
Professor, Department of Epidemiology

David Sack, MD; Advisor
Professor, Department of International Health

Kawsar Talaat, MD
Assistant Professor, Department of International Health

Clive Shiff, PhD
Associate Professor, Department of Molecular Microbiology and Immunology

Alternates:

William Greenough, MD
Professor, School of Medicine

Maria Merritt, PhD
Associate Professor, Department of International Health

DEDICATION

To my grandparents,
For teaching me everything important.

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LIST OF ABBREVIATIONS AND ACRONYMS

Acute Watery Diarrhea	**AWD**
Alkaline Peptone Water	**APW**
Antimicrobial Resistance	**AMR**
Case Fatality Rate	**CFR**
Comprehensive-Targeted Intervention	**CTI**
Department of Health Services	**DoHS**
District Public Health Office	**DPHO**
Epidemiology and Disease Control Division	**EDCD**
Female Community Health Volunteer	**FCHV**
Health Management Information System	**HMIS**
Institutional Review Board	**IRB**
Multi-Locus Variable-Number Tandem-Repeat Analysis	**MLVA**
National Public Health Laboratory	**NPHL**
Oral Cholera Vaccine	**OCV**
Phosphate Buffered Saline	**PBS**
Polymerase Chain Reaction	**PCR**
Rapid Diagnostic Test	**RDT**
Rapid Response Team	**RRT**
Thiosulfate Citrate Bile Salt Sucrose	**TCBS**
Vibrio cholerae	***V. cholerae***
Village Development Committee	**VDC**
Water, Sanitation and Hygiene	**WASH**
World Geodetic System	**WGS**
World Health Organization	**WHO**

CHAPTER ONE

LITERATURE REVIEW

1.1 Cholera

1.1.1 The disease

Cholera presents clinically as the rapid onset of acute watery diarrhea, often referred to as rice-water stool, and vomiting. Without treatment, the disease can progress rapidly and lead to death from severe dehydration in as little as four hours from onset of symptoms.[1] Prompt treatment, preferably with oral rehydration solution, can reduce the case fatality rate dramatically by limiting the loss of electrolytes, which would otherwise lead to lowered blood pressure and shock.[2,3] Severe cases may require intravenous fluids to keep up with the body's rapid loss of fluid. Antibiotics shorten the duration of illness and shedding, and should be used for treatment of cases that present to a health facility.

1.1.2 The organism

Cholera is caused by ingestion of the Gram-negative, rod-shaped, facultatively anaerobic bacterium, *Vibrio cholerae*.[4] Serotypes of *V. cholerae* are distinguished from one another by the O-antigen of the outer membrane's lipopolysaccharide (LPS).[2] Out of the 200+ serotypes of *V. cholerae* in existence, epidemic cholera disease is caused by the two toxin-producing strains O1 and O139 (non-O1/non-O139 *V. cholerae* may still be pathogenic).[5,6] *V. cholerae* O139 began causing disease in the 1990's, but has been

largely replaced by the O1 serotype. Serotype O1 is further divided into two biotypes, classical and El Tor, which can be differentiated based on their phenotypic properties.[7] The classical biotype of the O1 *V. cholerae* was associated with the earlier pandemics, although it has been reported more recently in Mexico as well during a 2012 outbreak in Nepal.[8-11] Biotype El Tor has now largely replaced the classical, and is considered the cause of the seventh pandemic. Both classical and El Tor biotypes can be further characterized into either Inaba or Ogawa serotypes based on the antigens they produce.[2]

Two virulence factors, cholera toxin and toxin co-regulated pilus, are responsible for cholera disease.[12] Toxin co-regulated pilus plays an integral role in colonization of the human intestines by *V. cholerae*.[13] It has also been shown to play a specific role in attachment and bacterial cell protection through biofilm formation within the intestines of the human host. Cholera toxin is an enterotoxin that consists of six subunits, one active subunit (A) and five binding subunits (B) (**Figure 1**).[2] Together the B subunits bind to receptors in the mucosa of the small intestine, while the A subunit enters the cells and activates a process resulting in the discharge of large amounts of fluid into the intestine.[14] This excess fluid over-powers the intestines absorption abilities and leads to the characteristic watery diarrhea.

1.1.3 The disease burden globally

Cholera has caused seven pandemics since 1817, with the seventh currently on-going.[8] A lack of adequate disease surveillance systems in the countries most often plagued by

cholera has led to an inability to precisely define the global burden. Distinguished from other diarrheal diseases by its severity, timely delivery of appropriate therapy is highly successful at preventing death. Despite effective treatment options, out of the approximately 1.3 billion people at risk of cholera in endemic countries, an estimated 2.86 million people contract cholera each year, with around 95,000 deaths.[15] Eight countries are estimated to see more than 100,000 cases per year, with the majority of these high-burden countries in Africa. The disease disproportionately affects disadvantaged groups and recent outbreaks have been a constant reminder of this. The 2010 outbreak in Haiti has become a seven-year tale with 807,000 cases and over 9,500 deaths.[16] The most recent (2017) outbreak in war-torn Yemen has already reached 500,000 cases and 2000 deaths, with estimates of 5000 new infections per day. While these outbreaks have been widely discussed among the global health community and news media, they are far from the extent of the problem, with at least 14 countries reporting deadly outbreaks from June 2015 to June 2016 alone.[17]

1.1.4 The disease burden in South Asia

A total of 51 countries are classified as endemic for cholera according to a 2015 article on the global burden of the disease.[15] Six of these countries are in South East Asia, including Nepal and its neighbors, China and India. Cholera is considered to have been born in this region, with the first written description of the disease in India between 500-400 BC, and the first of the seven pandemics beginning in the Ganges river delta of India in 1816.[18,19] In fact, all of the pandemics began in this region, with only the current pandemic originating outside of India in Indonesia. South Asia is estimated to have over

818,000 cases of cholera annually, with around 24,500 deaths.[15] However, these estimates are a result of statistical modeling as most cholera cases in this region, and around the world, go unreported. While this is in part a surveillance issue, there is often a large economic disincentive to report as well particularly in regard to trade and tourism. The high number of cases from this region is likely attributable to their high number of risk factors for the disease, including high population density, extreme poverty, lack of development, and environmental factors such as monsoon flooding.[20]

1.2 Nepal

1.2.1 The country

Nepal is a land-locked country, bordered by China to the north and India to the west, south, and east. The country has a population of approximately 28.5 million and consists of five development regions, the far-western, mid-western, western, central, and eastern regions (**Figure 2**).[21] These regions are divided into 14 administrative zones, comprised of 75 districts; these are further broken down into village development committees (rural areas) and municipalities (urban areas), which are made up of wards. There are three distinct ecological zones: the mountains, the hills, and the terai. A small minority of the population resides in the mountains (around 7%), while the rest are split between the hills and terai.[22] Kathmandu Valley lies within the hill region, and is the most densely populated and highly urbanized area.

1.2.2 The health system

Nepal's Department of Health Services (DoHS), within the Ministry of Health, is divided into seven divisions (Planning and Foreign Aid, Family Health, Child Health, Epidemiology and Disease Control, Logistic Management, Human Manpower Institutional Development, and Leprosy Control) and five centers (Health Training, Health Education Information and Communication, Tuberculosis, Center for AIDS and STD Control, and National Public Health Laboratory) organized as shown in **Figure 3**. At the regional level there is the Regional Health Services Directorate (Hospitals, Training Center, Health Laboratory, Medical Store, and Tuberculosis Center). The Zonal Hospitals report to their respective Regional Health Services and oversee the district level services (District Health Office, District Hospital, and District Public Health Office). The Primary Health Centers within each district directs the Health Posts (and Sub-Health Posts). Community level health in Nepal consists of Female Community Health Volunteers (FCHVs), Trained Birth Attendants, Primary Healthcare Outreach, and the Expanded Program on Immunization Outreach.[23]

Cholera surveillance and reporting in Nepal has two avenues: reported cases, and cases that are laboratory confirmed (**Figure 4**). In most cases, a patient reporting to a local health post with five or more episodes of watery diarrhea will be reported as a cholera case to the district public health office (DPHO) and enter into the country's Health Management Information System (HMIS). Alternatively, that patient may report to a hospital in their district that is able and/or required to confirm the suspected cholera case in the lab. These lab-confirmed cases are reported to the Epidemiology and Disease

Control Division (EDCD), as well as the National Public Health Laboratory (NPHL). In 1998, a program of laboratory-based Antimicrobial Resistance Surveillance (AMR) began enlisting hospitals with adequate capacity to test for resistance in certain isolated pathogens. There is virtually no communication between these two avenues for cholera-case reporting, which leads to contradictory descriptions of the true burden of disease.

1.2.3 The history of cholera in Nepal

Epidemics of cholera in Nepal can be traced back to at least 1823, when the disease was commonly attributed to "the influence of Saturn and other planets" and the "evil eye."[24] In 1885, Gimlette described a large outbreak, beginning in May in the Kathmandu Valley, with between 9 and 10 thousand deaths.[24] Outbreaks continued to occur yearly in the Valley and in 1958 the first bacteriologically confirmed epidemic was reported in Kathmandu to the World Health Organization Regional Office for South-East Asia. In 1961 cholera was first described as endemic in Nepal.[25] By 1991, cholera could be found in all five development regions in the country and was a routine contributor to the "annual monsoon-season gastroenteritis outbreak."[26] A yearlong study elaborated on the seasonality of cholera in the country, detecting no cases from December to May, with the highest caseload in August.[27] At least one study has reported an outbreak outside of the typical season, in October-November.[28] However, it is widely accepted that the rainy summer months in Nepal constitute the cholera season.

There have been a multitude of more recent published reports of outbreaks around the country. A 1992 outbreak in the Bhutanese refugee population led to the first molecular

description of a Nepalese strain, *V. cholerae* O1 biotype El Tor serotype Ogawa.[29,30] A single case of *V. cholerae* O139 was reported during a 1997 outbreak in Kathmandu, although a Nepalese O139 strain without direct connection to an outbreak had been reported previously.[31,32] In a 1996 paper on diarrheal disease in Nepal, two additional serotypes, Inaba and Hikojima, were identified in children under 14.[27] These serotypes were reported again in 2007 based on data from Nepal's National Public Health Laboratory.[33] Inaba serotype appeared intermittently as a small proportion of isolates for many years, but has not been detected since 2007.[34] The 2012 Kathmandu outbreak was the first report of the classical biotype in Nepal, with no instance of the El Tor biotype.[9,35] Overall, El Tor Ogawa has been the most commonly published biotype in the country.

A brief report in 1996 was the first to discuss antimicrobial resistance and noted reduced sensitivity to nalidixic acid, co-trimoxazol, ampicillin, and cephalexin.[36] Two years later the Ministry of Health in Nepal, in collaboration with the International Centre for Diarrhoeal Disease Research, Bangladesh, established an AMR Surveillance program. This program began as nine laboratories (now expanded to 22), reporting on five pathogens, one of which was *V. cholerae*.[37] In 2004, cholera isolated during an outbreak in Kavre district was found to be completely resistant to co-trimoxazole, but sensitive to all other antibiotics tested.[38] Another study conducted that year found resistance to nalidixic acid as well.[39] By 2008, an outbreak in Kathmandu (and later in Saptari and Jajarkot) produced isolates that were 100% resistant to furazolidone in addition to co-trimoxazole and nalidixic acid.[33,40-43] A later study identified this

resistance pattern as early as 2005.[44] Resistance to trimethoprim, sulfamathoxazole, and decreased susceptibility to ciprofloxacin was reported in 2010 and to streptomycin in 2012.[45,46] Multi-drug resistant *V. cholerae* has also been identified in Kathmandu's sewer system.[47] The variation in resistance profile results in the need for continuous monitoring to ensure effective drugs are being used when necessary.[46]

The vast majority of published outbreaks of cholera in Nepal are in Kathmandu. While it could be argued that Kathmandu simply has the greatest capacity for case detection, they also have dealt with an influx of people from rural areas, impacting the city's ability to provide safe drinking water and proper sanitation.[34] In the aftermath of the April and May 2015 earthquakes in Nepal, access to a water supply already considered unsafe decreased dramatically.[46,48,49] This, along with the damage sustained by 90% of the health facilities in the highly affected rural areas, has increased the country's risk for an outbreak of cholera.[49,50] Although Nepal made it through its first post-earthquake monsoon season with no increased incidence of cholera, the danger has not yet passed.[51]

1.3 Tools in Cholera Diagnosis and Prevention

1.3.1 Rapid diagnostic test

In the field where laboratory capacity is limited, rapid diagnostic tests (RDT) can be used to diagnose O1 and O139 *V. cholerae*.[52] The dipstick test uses monoclonal antibodies specific to the LPS of O1 or O139 serotypes to trigger a visual response in the presence

of the organism, similar to a pregnancy test.[53,54] The dipstick can be inserted directly into a stool sample, which has been mixed into a small amount of buffer solution, and results are visible in 15 minutes. Colored bands indicate whether the sample is positive for O1, O139, or neither, in which only the control line is visible. In comparison to culture, the rapid diagnostic test is about 71-77% specific in the field.[55-58] Sensitivity is much greater, documented at around 90%. Specificity is consistently higher, over 90%, with sample enrichment.[53,56,57,59,60] In the enhanced dipstick method, specimens are enriched in a selective media, alkaline peptone water (APW), for six hours prior to testing the broth with the dipstick.[59,60]

One potential limitation to the use of RDTs is their use in conjunction with the oral cholera vaccine. The vaccine can be used in a reactive manner during an outbreak to prevent the spread of disease. Since the cholera vaccines used today contain killed, whole cells of *V. cholerae*, RDTs may produce positive results from the stool of vaccinated individuals who are free of cholera disease. While this phenomenon has been documented, the period of risk for false positives has not been studied extensively.[61]

1.3.2 Filter Paper Specimen Storage

Cholera occurs most often in countries with limited resources and laboratory capacity, especially in rural areas. Many cases go undiagnosed or unconfirmed due to a lack of cold chain or media necessary to transfer samples to a better-equipped reference laboratory for processing. Debes et al. showed that bacterial DNA from stool samples could be preserved on filter paper during diarrheal disease surveillance, eliminating the

need for resource-demanding storage and making transfer to a reference laboratory for molecular processing much simpler.[62] In a country like Nepal where little capacity for advanced methods exists outside of the capitol city, this method could be a huge step towards better surveillance and confirmation of the national cholera burden.

1.3.3 Molecular Tools

The typical standard for confirming the presence of cholera in the stool of a patient in countries like Nepal is bacterial culture on selective media. However, this method is not 100% sensitive and sensitivity decreases if there are delays in transport of the specimen to the microbiological laboratory, as occurs commonly from remote areas of Nepal. For laboratories with capacity for molecular testing, confirmation by polymerase chain reaction (PCR) is a more reliable gold standard. This method utilizes a series of multiplex (multiple primer) PCR amplifications to determine the presence of cholera DNA in the stool. These primers are designed in sequence to target Vibrio species, distinguish toxigenic from non-toxigenic cholerae, and finally identify the serogroup. Since only DNA is required, the cholera organisms do not need to be alive for this detection method, making it an ideal alternative to culture when stool samples cannot be obtained for processing in a timely manner.

To further distinguish the *V. cholerae* strains identified, a method known as multi-locus variable-number tandem-repeat analysis (MLVA) is used. MLVA works by identifying short DNA sequences that repeat in tandem at specific, pre-defined loci specific to the pathogen of interest.[63] The number of repeats make up a “DNA fingerprint” and can be

compared with a reference library to differentiate the strains. This method is a particularly useful epidemiologic tool in outbreak situations to determine how related strains are, and how that may change over time and space.

1.3.4 Oral Cholera Vaccine

Cholera vaccines have been used in humans as far back as 1885.[64] Up until the 1970's attenuated cholera vaccines were injected, or parenteral, until a study showed that the vaccines performed poorly, with only 50% protection for less than six months.[65,66] In 1985 the first oral vaccine went to trial, but it was not until 2010 that oral cholera vaccines (OCV) were recommended by the World Health Organization (WHO) for their safe and easy administration, higher acceptability, and attention to the idea of mucosal immunity.

There are currently four licensed killed vaccines for cholera prevention: (i) Shanchol, which contains killed whole cells of *V. cholerae* serogroups O1 and O139; (ii) Dukoral, containing killed whole cells of *V. cholerae* serogroup O1 and recombinant B-subunit of cholera toxin; (iii) mORC-Vax, which is very similar to Shanchol; and (iv) Euvichol, killed whole cells of *V. cholerae* O1 (Classical and E1 Tor biotypes) and *V. cholerae* O139.[67] Dukoral, Shanchol, and Euvichol were prequalified by the World Health Organization in 2001, 2011, and 2015, respectively.

Shanchol has been shown to confer 58% cumulative protection with two doses to recipients on average for at least three years.[68-70] This immunity extends the time

horizon to observe the benefits of vaccination. Therefore, vaccine can confidently be given to any at-risk population, without the need to pinpoint exactly when an outbreak will occur. The vaccine also induces significant herd protection, but more research needs to be done into how to maximize this benefit. While no studies have been published with one-dose efficacy for longer than 6 months as the primary endpoint, a one-dose regimen does provide significant short-term protection.[71,72]

More than 11.5 million doses of OCV have been administered worldwide.[73,74] In 2013, WHO established a stockpile of OCV and issued the following statement: "Given the availability of two oral cholera vaccines and data on their efficacy, field effectiveness, feasibility and acceptance in cholera-affected populations, immunization with these vaccines should be used in conjunction with other prevention and control strategies in areas where the disease is endemic and should be considered in areas at risk for outbreaks." Requests exceeded supply for the first time in 2015, and have been doubling every year since 2013.[75] This increase may be due to the more visible and accessible supply, but could also be the result of increasing amounts of information in the literature on effectiveness and feasible delivery strategies.[76]

OCV is typically delivered through campaigns, rather than through a routine immunization program. Rather than vaccinating entire countries through national campaigns, it is given in specific areas that are deemed to be at highest risk. There are three general times in which OCV will be used, in an endemic area for prevention, to prevent outbreaks in humanitarian emergency settings, and in response to an outbreak

that is already underway. While it has been the standard approach in all three scenarios to identify an area of high risk, such as a refugee camp or municipality, and vaccinate all members with two doses of vaccine, there has recently been buzz in the field surrounding a ring vaccination approach to outbreak response.[77,78] This approach builds upon the idea that those at highest risk of disease are individuals that live in close proximity to a case. This is another method of determining a high-risk area, which could be ideal in settings where little is known about the epidemiology of cholera or when vaccine is in short supply.

OCV is not intended to be the sole intervention to prevent cholera mortality. Where it was once considered competitive, it is now considered important that OCV be integrated with other interventions, such as improved case management, water, sanitation and hygiene (WASH), and health communications.[76,79] Where and when to use OCV depends strongly upon surveillance, which is generally lacking in most cholera endemic areas.[49] Combining with other measures ensures some level of protection for the population, specifically for a reactive strategy where intensive WASH could provide protection during the 4 – 7 days it will take for OCV to become protective.

1.3.4 OCV in Nepal

A cholera outbreak in the Central Region of Nepal, along the border of Rautahat and Makawanpur districts, began on April 30, 2014. The affected area was isolated, rural, and suffered from poor water and sanitation. When a social mobilization campaign to inform residents in the area about the importance of WASH strategies in preventing infection

failed, the EDCD of the DoHS in Nepal made the decision to conduct an immunization campaign in the region. A plan for conducting the campaign, and a request for vaccine was sent to the Global Cholera Vaccine Stockpile Unit at the WHO. The application was successful and 36,000 doses of OCV (Shanchol) were granted to the country. Two rounds of vaccination took place between September 12-13, 2014 and September 26-27, 2014. Out of the 36,000 doses procured, 16,000 were used during the campaign, which resulted in an administrative coverage rate of 72%.

In the aftermath of the April and May 2014 earthquakes, a second vaccination campaign was carried out in the Central Region in the district of Newakot. Unlike the previous reactive campaign, this campaign was preemptive, as Newakot was highly affected by the earthquake. Camps of internally displaced persons, as well as villages at high risk of outbreak were targeted. Two doses of vaccine were offered, with the first round of immunizations from August 8-12, 2015 and the second round from August 30th – September 3, 2015. The remainder of the country's OCV stockpile was used during this campaign (20,000 doses). The EDCD estimates an administrative coverage rate of 95%.

Banke district in Western Nepal has consistently reported cases of cholera for the past five years and, combined with a high proportion of the population that lacks access to safe water, sanitation, hygiene and health services, it is an area at high risk for continued outbreaks. Due to a generous donation from the Rotary Global Grant Fund, a total of 55,000 doses of OCV were given for a vaccination campaign in three areas of Banke district. Banke district has 46 Village Development Committees (VDCs) and one

municipality, with a total population of 0.49 million people. The total selected population for the targeted OCV campaign was nearly 27,000 individuals residing in the VDCs Sonapur and Udarapur, and ward five of Nepalgunj municipality. An estimated 24,000 people were vaccinated with two doses during this campaign and the administrative two-dose coverage was estimated to be over 90%.

1.4 Rationale

The likelihood of a quick recovery from the earthquake-imposed damage incurred in Nepal is unlikely, largely due to the extent of the loss of infrastructure.[80] Between 660,000 and 1.3 million people suffered damage to their primary water sources, and sanitation support is needed for 850,000 to 1.7 million. This is on top of the 945 heath facilities that have either partially or totally collapsed. While the country escaped its first monsoon season with reports of only a small outbreak in the Kathmandu Valley, the risk of cholera remains great in the Valley. With little reconstruction, the situation remains bleak regarding water and sanitation in the most highly affected districts. This, combined with a lowered guard and a lack of awareness of risks due to the lack of a large outbreak last season, could lead to a larger outbreak and a delayed response.

A review of the literature on cholera in Nepal revealed that while the disease is endemic and occurs nearly every year in the Kathmandu Valley, exactly where it will occur is difficult to predict. This is partially due to a fragmented surveillance system, with no solid body of evidence to support advanced preparation. As a foundation for this dissertation work, a retrospective analysis of the cholera burden in Nepal from 2005 to

2014 was performed in an effort to shed light on the state of the surveillance system, how it functions in the country, and paint a clearer picture of which areas of the country are at greatest risk of outbreaks, historically. Data was collected on both reported and confirmed cholera cases. For reported cases, case counts and line lists were collected from district public health offices around the country representing all five development regions and three ecological zones. A meeting of the Steering Committee for Enteric Diseases was held on April 15th, 2015 where it was decided that data would be collected from 25 districts on the basis of frequent outbreaks and high incidence of severe diarrhea cases as reported in the DoHS Annual Reports beginning in 2011. Confirmed case counts were collected from 17 of the country's 18 AMR sites, which included both public and private institutions, as well as two referral hospitals.

Over a four-month period, study data was collected retrospectively from 22 districts and 12 hospitals. A total of 26,947 cases of cholera were reported from these 22 districts from 2005 to 2014 with an overall case fatality rate (CFR) of 1.2%. Seven of 22 districts reported no cases during that ten year period, six of which (Taplejung, Illam, Jhapa, Sunsari, Okhaldhunga, Bhaktapur, and Darchula) had reported cholera cases in the DoHS annual reports.[81]

Information was collected on 2,140 inpatient cases with severe diarrhea from 13 of the 19 hospitals the study planned to visit. Out of these cases, 628 had a provisional diagnosis of cholera and only four had cholera listed as the final diagnosis. None of the cases were confirmed by stool culture.

Figure 5 shows the total number of cases and deaths reported per year from all 22 districts combined, in addition to the CFR. No data was available for 2005, and no cases were reported in 2006 or 2007. While 2009 and 2014 both had large numbers of cases, it is important to note that in 2014 over 96% of the cases were from a single district, Rukum.

The location of cases varied from year to year, but the majority of cases were reported in the Mid-western region of the country. It is highly likely that these cases are only the tip of the iceberg. In a retrospective study on the incidence of cholera in Uganda, it was assumed that the cases detected were only 25% of the true total, as most diarrheal cases (in Uganda as well as Nepal) are treated at home.[82] The significant amount of data missing at the hospital and district level may reveal that the surveillance data in Nepal is largely incomplete and further masks the true cholera burden. If this is the case, the same could likely be concluded about cholera mortality, as deaths that occur outside the hospitals/health posts would go unrecorded.

The WHO recommends confirmation of the clinical diagnosis in a small fraction of cases at the beginning of an outbreak. However, there was a lack of lab confirmed cholera cases at the 16 AMR sites visited for data collection even in districts with cholera cases reported. It seems that while the AMR sites have the laboratory capacity to confirm cholera cases through stool culture on paper, this is not happening in practice.

This review assisted in identifying the limitations of the cholera surveillance system, but leaves the question of how best to approach cholera surveillance and prevention in light of those weaknesses. As outlined above, the global supply of OCV is incredibly limited as compared to the global at-risk population. While mass vaccination of the at-risk population seems reasonable given the increased risk post-earthquake, this population in the country is much greater than the available vaccine. By designing an intervention that focuses on halting an outbreak early rather than preventing one entirely, it may be possible to avoid more morbidity and mortality. A study in Matlab, Bangladesh showed that those living closest to a case (within 50 meters) had 36 times the chance of becoming infected with cholera than those living in other areas of the community.[78] This risk was highest during the first three days after the index case was identified. A reactive vaccination strategy, when partnered with other key cholera prevention measures, forms the backbone of a comprehensive-targeted intervention (CTI) approach to cholera control and has the potential to halt the spread of cases if deployed rapidly. Founded on a strengthened hospital-based surveillance system, the CTI approach combines pointed health behavior messaging with traditional WASH interventions and a single-dose (87% short-term effectiveness) OCV campaign to prevent the spread of cholera once it strikes.[71] Demonstrating feasibility and effectiveness of such a strategy would provide a new cholera control method for Nepal and other countries in post-disaster and high-risk situations.

1.5 Specific Aims

The overall goal of this dissertation was to assess the feasibility of a comprehensive, targeted response to a cholera outbreak in Kathmandu Valley, Nepal in a way that simultaneously generates data that can be used to better understand the epidemiology of the disease.

1.5.1 Specific Aim 1

Evaluate the feasibility of a comprehensive, targeted intervention that employs a reactive ring approach for preventing the spread of cholera in Kathmandu Valley, Nepal

1.5.2 Specific Aim 2

Differentiate clinical cholera isolates from an outbreak in Kathmandu, Nepal using multi-locus variable-number tandem repeat analysis to explain the genetic epidemiology of the disease in the Valley

1.5.3 Specific Aim 3

Determine the spatiotemporal relationship of cholera cases during the 2016 outbreak in Kathmandu, Nepal to predict the maximum usefulness of oral cholera vaccine in this setting

1.6 Chapter One Figures

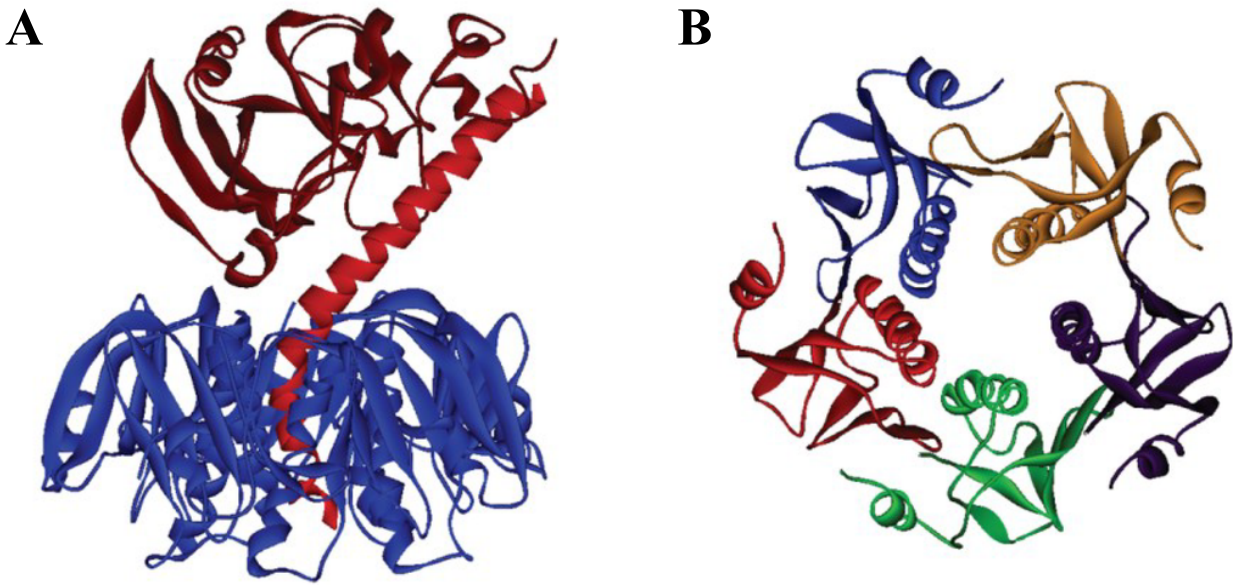


Figure 1.1 Structure of the cholera toxin. Panel A shows the A subunit in red and the B subunit in blue. Each monomer of the B subunit can be seen in a different color in Panel B. Image Source: Baldauf et. al., 2015.[83]

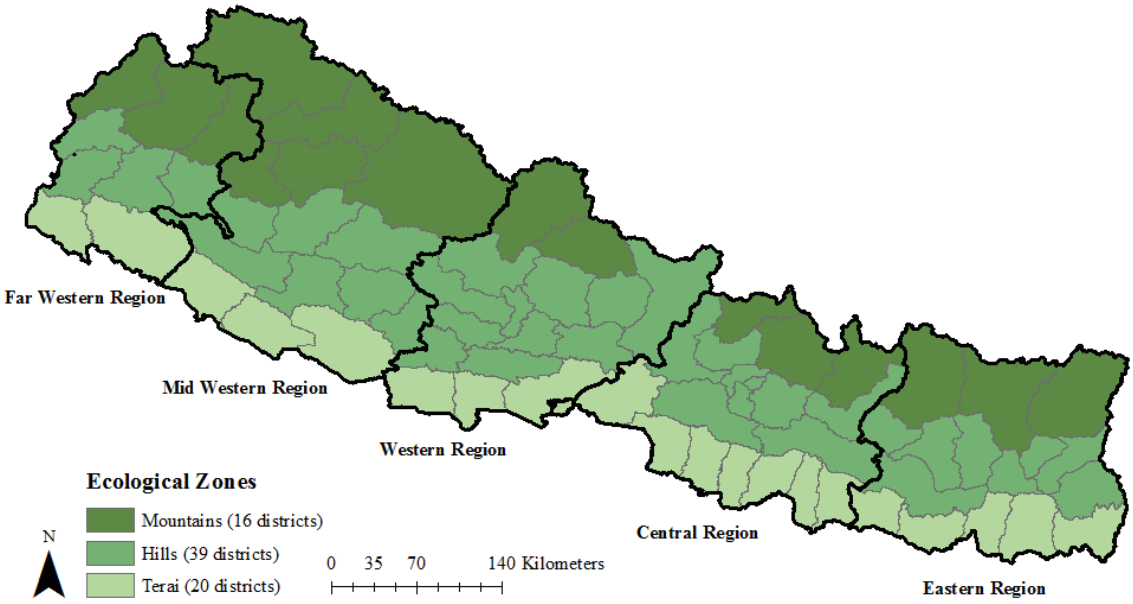
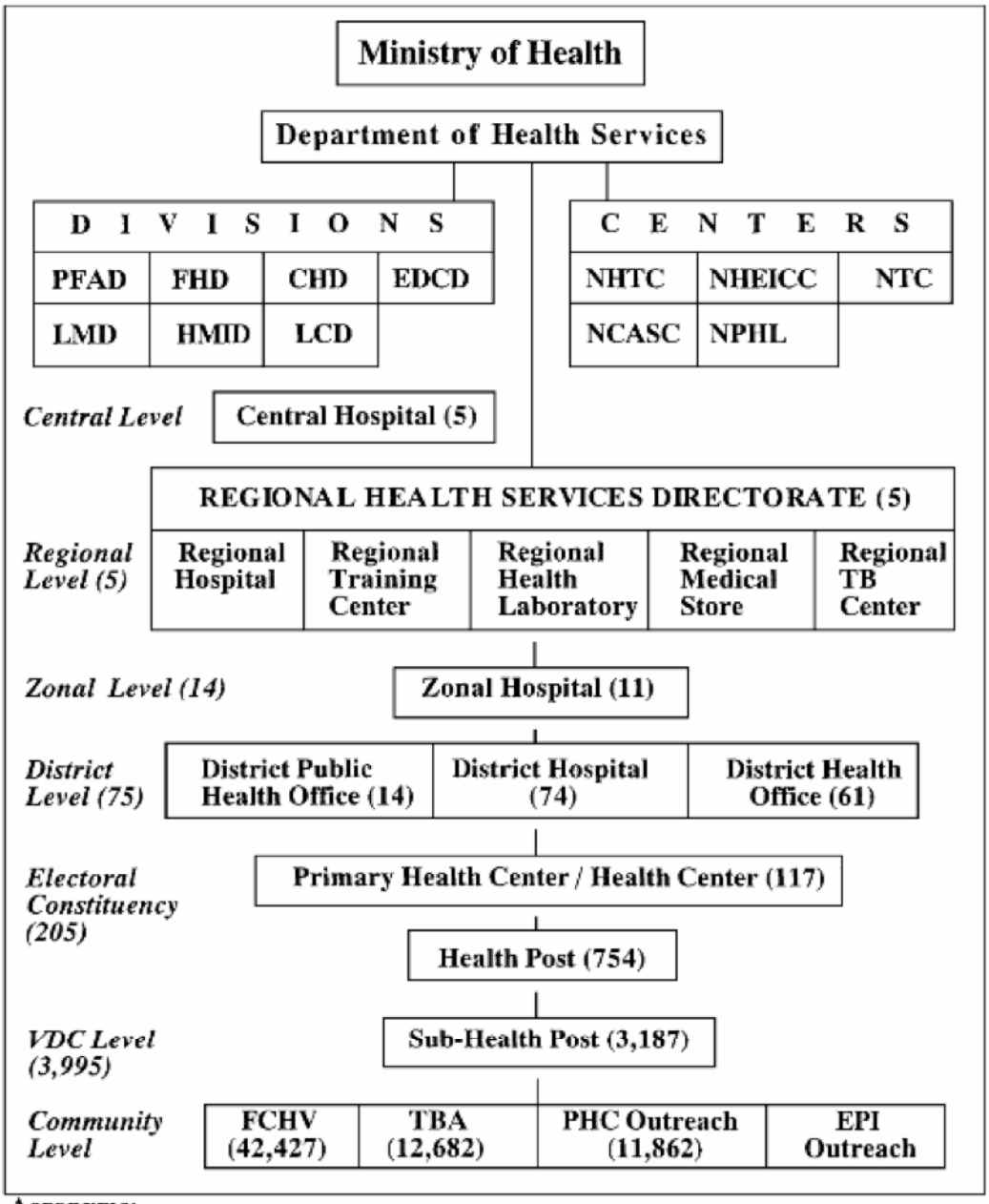


Figure 1.2 Development regions and ecological zones of Nepal. The study area is located in the hills of the central region.



Acronyms:

PFAD	Planning & Foreign Aid Division	NTC	National Tuberculosis Center
FHD	Family Health Division	NCASC	National Center for AIDS & STD Control
CHD	Child Health Division	NPHL	National Public Health Lab.
EDCD	Epidemiology & Dis. Control Division	FCHV	Female Community Health Volunteer
LMD	Logistic Management Division	TBA	Traditional Birth Attendant
HMID	Human Manpower Institutional Develop.	PHC	Primary Health Care
LCD	Leprosy Control Division	EPI	Expanded Program on Immunization
NHTC	National Health Training Center		
NHEICC	National Health Education, Information & Communication Center		

Figure 1.3 Organization of the health system in Nepal. Image Source: Rai et.al., 2001.[23]

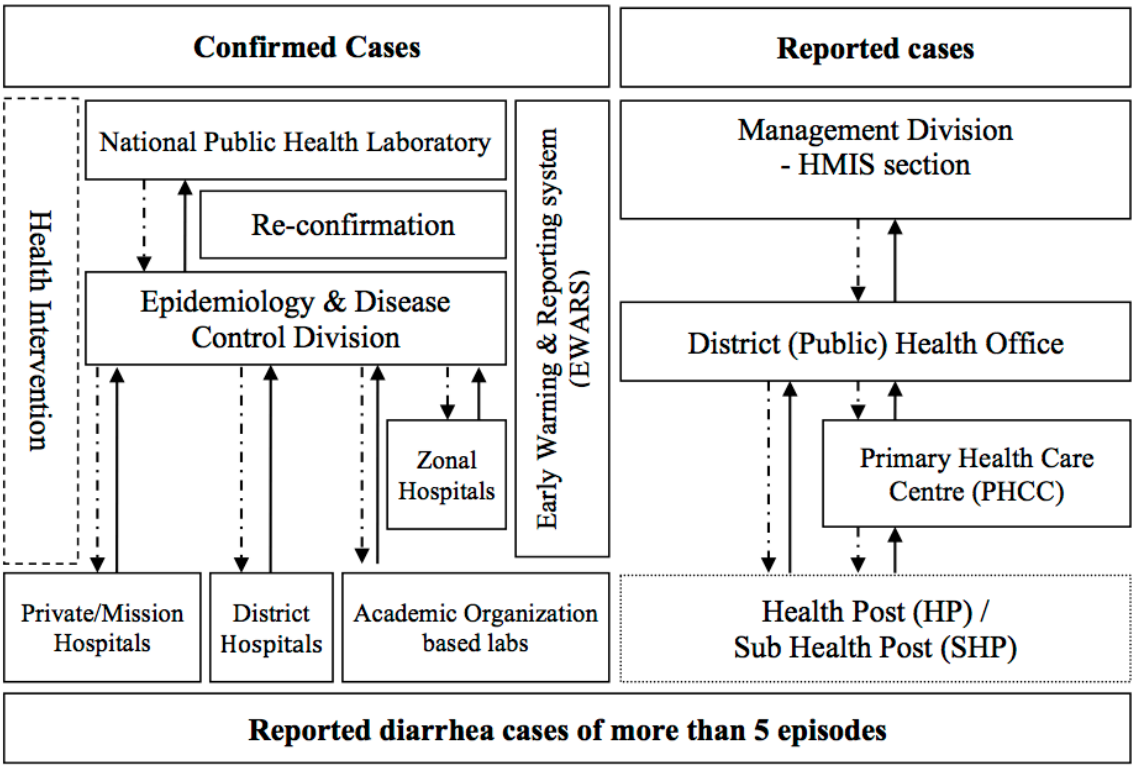


Figure 1.4 Framework for cholera surveillance and reporting in Nepal. Solid arrows indicate the flow of information from the health facilities to the national level. Dashed arrows represent reporting from the government to the periphery on surveillance efforts. Note that there is no overlap between the two avenues, resulting in conflicting reports of disease burden.

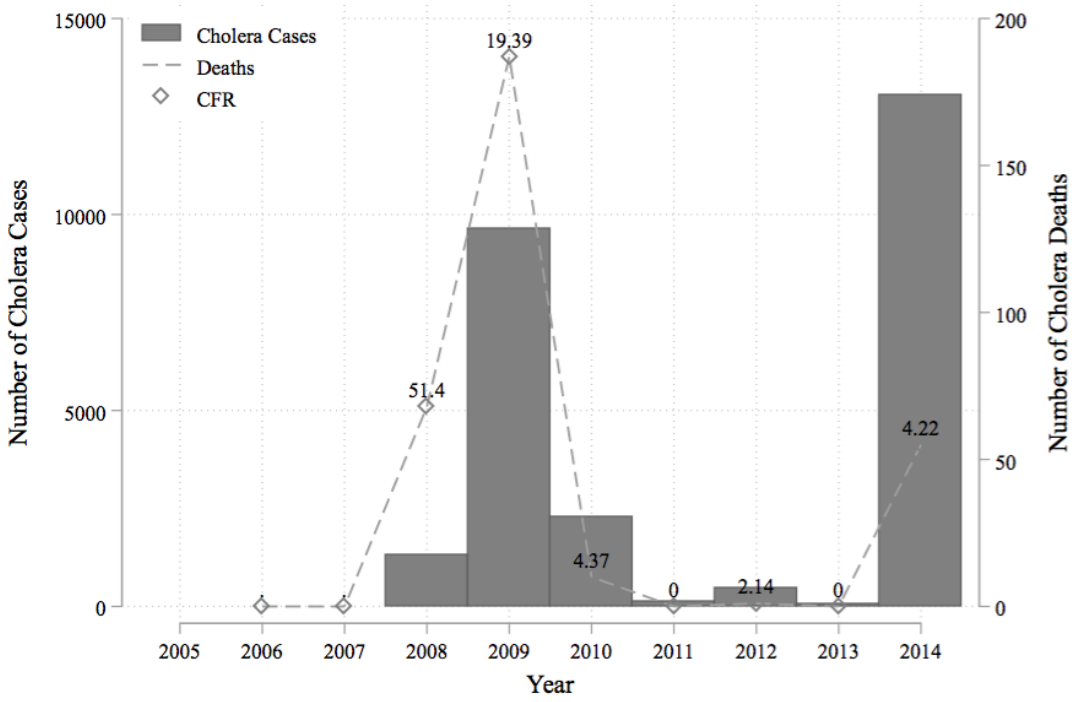


Figure 1.5 Annual number of cholera cases and deaths from 2005 to 2014. Cases were reported from 22 districts as part of a retrospective study of cholera in Nepal. Diamonds indicate the case fatality rate for that year. No data was available for 2005.

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CHAPTER TWO

PAPER ONE

FEASIBILITY OF A COMPREHENSIVE TARGETED CHOLERA INTERVENTION IN KATHMANDU VALLEY, NEPAL

Mellisa Roskosky[1], Bhim Acharya[2], Geeta Shakya[3], Kshitij Karki[4], Deepak Bajracharya[4], David Sack[1]

[1] Johns Hopkins University, Baltimore, MD, USA

[2] EDCD, Department of Health Services, Kathmandu, Nepal

[3] NPHL, Department of Health Services, Kathmandu, Nepal

[4] Group for Technical Assistance, Kathmandu, Nepal

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2.1 Paper One Abstract

2.1.1 Background

Cholera is endemic in Nepal and the vast majority of published outbreaks of cholera in the country have been in Kathmandu. Studies have demonstrated a much higher risk of cholera in the immediate area around a case and that this increased risk persists for about 2 to 3 weeks. A Comprehensive Targeted Intervention (CTI) was designed and deployed in the neighborhoods of cholera cases in the Kathmandu Valley during 2016 with the intent to reduce rates among neighbors of the case. This was a feasibility study to determine if clinical centers, laboratories and field teams were able to cooperate and coordinate a response within 48 hours of hospital admission. We hypothesized that this strategy would be a cooperative and unifying method for cholera control in the area.

2.1.2 Methods

Daily line listings were requested from the 12 participating hospitals, and a single case initiated the CTI. A standard case definition was used: Acute watery diarrhea, with or without vomiting, in a patient aged one year or more. Rapid diagnostic tests and bacterial culture were used for confirmation. The strategy included hospital and household investigation of cases, water testing, WASH intervention, and health education. A single-dose of oral cholera vaccine using a reactive ring strategy was included in the plan, but vaccine could not be obtained in time for the summer 2016 outbreak. An intervention coverage survey was conducted 8 months post-monsoon season.

2.1.3 Results

Between June 1 and December 30, 2016, 169 cases of *V. cholerae* O1 were confirmed by bacterial culture. Average time to result of hospital culture was 2.4 days. On average, the CTI Rapid Response Team (RRT) was able to visit the household 2 days after the culture result was received from the hospital. 90.4% of household water samples were found unsuitable for drinking and 3 sources were positive for cholera. Coverage of WASH and health behavior messaging campaigns was 30.2% in the target areas. Recipients were 2.3 times more likely to have knowledge of cholera symptoms, treatment, and prevention.

2.1.4 Conclusion

This attempt at implementing CTI was successful in raising awareness and engaging stakeholders in both government and private sectors. While the RRT were able to investigate cases at the household within 48 hours of a positive culture result, the study identified several constraints that limited a truly rapid response. Using the information from this first year, we identified pathways that will be useful for future implementation of CTI which have been included in the country's national cholera control strategy.

2.2 Introduction

Cholera, caused by ingestion of the bacterium *Vibrio cholerae*, presents clinically as the rapid onset of acute watery diarrhea and vomiting. Without treatment, the disease can progress rapidly and lead to death from severe dehydration in as little as four hours from onset of symptoms.[1] Prompt treatment can reduce the case fatality rate dramatically by replacing lost fluid and electrolytes, which would otherwise lead to lowered blood pressure and shock.[2,3] A study in Matlab, Bangladesh showed that those living closest to a case (within 50 meters) had 36-fold higher chance of becoming infected with cholera than those living in other areas of the community.[78] This risk was highest during the first three days after the index case was identified. Similar results were seen in Kolkata, India, where an increased risk for cholera was seen within 25 to 50m of a case and persisted for a month.[77] These observations highlight that rapid detection of cholera cases is needed in order to react and prevent the spread of disease.

Cholera is endemic in Nepal, with an estimated 30,000 cases and 911 deaths per year during the monsoon months of May through September.[15] While the disease occurs nearly every year in the Kathmandu Valley, exactly where it will occur is difficult to predict. This is partially due to a fragmented surveillance system, with no solid body of evidence to support advanced preparation.

Despite much recent progress, the global supply of oral cholera vaccine (OCV) is incredibly limited as compared to the global at-risk population.[76] By designing an intervention that focuses on halting an outbreak early rather than preventing one entirely,

it may be possible to limit the transmission from index cases and reduce overall rates of disease by targeting the high-risk groups. A reactive vaccination strategy, when partnered with other important cholera prevention measures, forms the backbone of a comprehensive-targeted intervention (CTI) approach to cholera control and has the potential to halt the spread of cases if deployed rapidly. Founded on a strengthened surveillance system, the CTI approach combines pointed health behavior messaging with traditional water, sanitation and hygiene (WASH) interventions and a single-dose OCV campaign (estimated to have a short term effectiveness of 87%) to prevent the spread of cholera once it strikes.[71] Despite much recent progress, the global supply of OCV is incredibly limited as compared to the global at-risk population.[76] By designing an intervention that focuses on halting an outbreak early rather than preventing one entirely, it may be possible to reduce morbidity and mortality. Demonstrating feasibility and effectiveness of such a strategy would provide a new cholera control method for Nepal and other countries in post-disaster and high-risk situations.

2.2.1 Study Objective and Rationale

The Epidemiology and Disease Control Division (EDCD) of the Department of Health Services (DoHS) in Nepal adopted a CTI approach to cholera control for the 2016 monsoon season, expanding the role of the country's existing rapid response team (RRT) network. This is a feasibility analysis of that response. We hypothesized that a CTI strategy for controlling cholera in Kathmandu Valley would facilitate a cooperative and unifying method for cholera control and could potentially reduce transmission in this area. Deploying an early warning system and RRTs for cholera in post-earthquake Nepal

faces many challenges – poor disease surveillance, limited laboratory capacity, and loss of health infrastructure to name a few.[49] The main challenge lies in engaging stakeholders involved in cross-sectoral activities to work together and achieve a fast and effective response to a cholera outbreak once it has started.

2.3 Methods

This was designed as a feasibility study to determine if clinical centers, laboratories and field teams were able to cooperate in a manner that resulted in a CTI approach within 48 hours of a case reporting for treatment. Detailed records on timeliness were kept for each phase of the response and these were reviewed with the goal of answering specific questions regarding time to response (**Table 1**).

2.3.1 Intervention Summary

Enhanced hospital-based surveillance for cholera cases took place at 15 sites throughout the Kathmandu Valley, consisting of Kathmandu, Lalitpur, and Bhaktapur districts. Sentinel sites identified patients suspected to have cholera using a standard case definition: Acute watery diarrhea (AWD), with or without vomiting, in a patient aged one-year or more. Daily line listings of AWD cases were requested from each hospital, including zero-reporting, and a single suspected case of cholera initiated the CTI cascade. When such a patient was identified, the hospital was expected to perform a rapid diagnostic test (RDT) or culture for cholera and sent a stool specimen to the National Public Health Laboratory (NPHL), for culture confirmation and serotyping. Within the same day (approximately six hours), any positive RDT result was reported to the EDCD.

Once notified, the RRT was expected to travel to the neighborhood of the index case the same day (or the next day if necessary) to initiate the CTI intervention.

The complete CTI intervention included (**Figure 1**):

1. Hospital and household investigations of each case
2. An intensive water, sanitation, and hygiene (WASH) intervention to the case household and the first degree neighbors
3. Community-level WASH activities and health education messaging in the neighborhood surrounding the case in order to encourage safe water, safe food and hand-washing
4. Water testing for *V. cholerae* at each index household
5. An OCV campaign in the neighborhood of the index case, given within a 100m ring, to approximately 1000 persons, using a single-dose strategy

2.3.2 Case Investigation

Patients meeting the case-definition for cholera were expected to have their clinical information recorded at the hospital by the RRT in what was termed the hospital investigation. Data collected here focused solely on the index case and included a summary of demographic characteristics, signs and symptoms, and approximate address. This data was aggregated and used to generate daily situation reports for the ministry and other relevant stakeholders.

The RRT was then to be deployed to the home of the index case within 24 hours to conduct the household investigation. GIS locations of the homes were recorded to map the geographic distribution of the outbreak. If a family did not consent to a home-visit, or if the RRT was unable to contact the family, the approximate location of the household was mapped. Household investigation data included information on water sources, water treatment, history of diarrhea within the household over the last two weeks, history of food consumption and travel, and sanitation and hygiene behaviors of the entire household.

2.3.3 WASH Intervention

The family of the index case and their immediate neighbors were then to be targeted for a door-to-door awareness campaign aimed at educating the high-risk groups on the risk of cholera transmission and methods for prevention. These campaigns were primarily targeting households, however, schools and food vendors were included if they were in the target area. The RRT was expected to visit the households within 48 hours of the initial index case in that area. Households were to receive orientation and equipment for point-of-use water treatment. This included chlorine tablets, basic buckets for water storage and hand washing, and soap. They were also expected to receive education, both verbal communication and a flyer for future reference, on hand washing at critical times, food hygiene, and personal hygiene and sanitation.

2.3.4 Health Education Intervention

Female community health volunteers (FCHVs) were also expected to deliver messages to the broader community surrounding the index case on various cholera prevention strategies. These messages included boiling, filtering, or treating water with chlorine, basic hygienic food preparation, parasite prevention methods, and the importance of vitamin A supplementation. Messages were to be delivered in a variety of ways in order to maximize coverage. Booths were set up in community areas with high foot traffic and FCHVs handed out flyers and answered questions. Awareness rallies were held with banners displaying prevention methods. Presentations were given at meetings of community groups and schools, and radio messaging was projected from a vehicle (miking).

2.3.5 Water Sampling

Surface water samples (3 liters each) were collected from primary and secondary drinking water sources for the index case households, filtered through sterile gauze, and incubated in selective media (alkaline peptone water) for 24 hours.[84] The NPHL then performed culture analysis to preserve any *V. cholerae* isolates on thiosulfate citrate bile salt sucrose agar.

RRTs and FCHVs used a coliform presence/absence (H_2S) test kit, as a visual demonstration of water unfit for drinking.[85] This version of the test was developed by the local Environment and Public Health Organization and is a simple, low-cost method for detection of fecal contamination in water. Water is added to the vial to a specific fill

line, incubated at room temperature for 24-48 hours and is observed for a color change indicative of contamination. These vials were used at households, schools, and in the community on all water sources.

The NPHL also performed a quantitative test for coliforms, the Idexx colilert-18 test.[86] When coliforms metabolize colilert-18's ortho-nitrophenyl-beta-D-galactopyranoside, the sample turns yellow. Yellow wells were then counted and coliforms per 100mL were determined via a most probable number table provided with the test kit.

Total chorine tests were also performed on household water sources as well as for tankers supplying water to the community. These tests used the low-cost chemical orthotolidine, which turns yellow in the presence of chlorine.[87] Results of testing at the community level were shared with that community as well as solutions to any water quality issues identified.

2.3.6 OCV Intervention

A single dose of OCV was to be administered by the RRT using a door-to-door, reactive ring strategy in the neighborhood of the index case. The ring size to be used was chosen by the EDCD to be approximately 100 meters based on knowledge of population density in the Kathmandu Valley and a goal of vaccinating approximately 1000 people per ring. The RRT was expected to deliver vaccine to men and non-pregnant women greater than one year of age in target households within 48 hours of the index case presenting to the

health facility. Unfortunately, vaccine could not be obtained in time for the summer 2016 outbreak.

2.3.7 Follow up

As part of the post-CTI program monitoring and evaluation, a survey was conducted in each of the areas targeted for community-level WASH intervention. A field team visited each of the intervention areas and administered a simple questionnaire to households using a multistage cluster sampling method. A total of 400 households were targeted for the survey. First-stage clusters were the targeted wards, and the number of households sampled was proportionate to population size. For first-stage clusters larger than 20 households, an additional cluster was added within that ward, resulting in 30 total clusters. Second-stage clusters were chosen according to the World Health Organization vaccine coverage survey guidelines.[88] The survey asked residents if their home was visited by a WASH volunteer, if they heard the health messaging in their neighborhood, if they received specific information on health promotion from the volunteers, received water purification materials, etc. It also included an assessment of their ability to answer basic hygiene promotion, water purification, and cholera prevention questions.

2.3.8 Analysis

The number of cases over the course of the outbreak was graphed and cases were mapped by location of household using ArcGIS. Basic demographic characteristics of AWD cases, cholera cases, and survey respondents were described. Feasibility was measured through indicators of timeliness and quality of implementation (**Table 1**). Survey data was summarized as a coverage rate and served as a proxy measure of quality. Data was

stratified by whether or not a WASH intervention was received in that household and comparisons were tested for significance via chi-square test. Statistical analyses were performed using Stata version 12 (StataCorp, LLC, College Station, Texas, USA).

2.3.9 Ethical approval

Institutional Review Board (IRB) approval was obtained from the Johns Hopkins University IRB and the Nepal Health Research Counsel for the use of de-identified data collected during the campaign.

2.4 Results

A total of 2,207 cases of AWD were reported from the sentinel sites to the EDCD between June and November 2016 (**Table 2**). Of those AWD cases, 239 were classified as suspected cholera. In total, 169 cases were culture confirmed as *V. cholerae* O1 Ogawa (**Table 2; Figure 2**). Rapid diagnostic test results were compared to bacterial culture and resulted in a sensitivity and specificity of 90% and 70%, respectively. No cases of the serotype Inaba were seen. Male-female ratio was similar between both patient groups. Cholera cases were significantly younger than AWD cases by approximately ten years ($p<0.001$). The majority of cases were detected from the Kathmandu Valley. The geographic distribution of cholera cases can be seen in **Figure 3**. Over 70% of cases were reported from Lalitpur district (120/169).

An evaluation of the speed with which the CTI was implemented can be seen in **Figure 4**. The average time from hospital admission to hospital laboratory results was 3.1 days (sd: 1.8; range 1 to 8 days). Average time from hospital admission to NPHL culture confirmation was 5.2 days (sd: 1.9; range 1 to 12 days). RRTs interviewed a total of 132 of confirmed cases in the hospital (78% of total cases) and out of those cases 92 household investigations were performed (54% of total cases). On average the RRT was able to visit the household 1.7 days after the culture result was received from the hospital (sd: 1.4; range: 0 to 6 days). It took an average of 4.0 days from hospital admission to household investigation (sd: 2.3; range 1 to 17).

Water samples were collected from all case households that were investigated and 90.7% (117/129) of those household water samples were found unsuitable for drinking based on coliform count (greater than 1 coliform per 100 ml). Three household water sources were positive for *V. cholerae* O1 Ogawa. Only 8.5% of drinking water samples tested had detectable levels of chlorine (10/118).

WASH and health behavior messaging campaigns were conducted in 18 areas of Lalitpur and Kathmandu districts. On average these campaigns happened 9 days after the initial case in that area was admitted to the hospital (sd: 6.8; range: 0 to 37 days). A total of 394 households were surveyed (**Table 2**), of which 119 reported hearing WASH messaging during the monsoon season for a coverage rate of 30.2%. When asked which messaging was heard, survey respondents were most likely to report hearing about the importance of water purification and most often via miking or a household visit (**Table 3**). The results

of the knowledge assessment can be seen in **Table 4**. While most respondents had heard of cholera and the importance of safe water, less than half thought that hand washing was important in prevention of disease. Univariate logistic analysis showed that those who had received WASH messaging were 2.5 times as likely to have heard of cholera (95% CI: 1.21, 5.08). Adjusting for age, sex, and education attenuated this association and the adjusted odds ratio was 2.33 (95% CI: 1.01, 5.37).

2.5 Discussion

The initial pilot of the CTI program in Kathmandu Valley suggests that this type of cholera control approach is feasible in an urban, developing country setting. Despite this being the first time such an approach has been used in the country, the program resulted in a heightened awareness of cholera and AWD in the Kathmandu Valley among government officials, hospital staff, and local NGOs. This increased awareness played a large role in the ability of the RRTs to mount a comprehensive response, rather than compartmentalized responses at each level of the health system. Historically, divides between clinicians, laboratory staff, and the government responders have existed in Nepal. Clinicians treat patients based on clinical symptoms and they are often discharged before laboratory results are available. Combined with the very little information collected on these cases at admission, this scenario has made it very difficult to follow up on cases in the past. This implementation of the CTI approach shows the potential to alleviate these issues, as participants at every level were required to communicate results effectively in order to remain within the established guidelines. Flow of information was

evident from hospital admission, and was successfully translated into household-level and in some areas community-level responses.

In addition to ensuring communication between historically disparate stakeholders, the evaluation of the CTI approach enables us to establish a baseline for time-to-response. Diagnostic capacity is lacking at the hospital level, and while some hospitals do have the capacity to perform culture confirmation of cholera, the time required to receive culture results diminishes the effectiveness of a response. RRTs were able to respond to cases within an average of 4 days, but three of those days were typically spent waiting for a culture result. This highlights the need for an expansion of rapid diagnostic testing at the hospital level for surveillance and response purposes. The use of point-of-care RDTs would simplify all levels of the cholera surveillance and response system by allowing the laboratory staff to provide clinicians with a rapid diagnosis, and the medical recorder with a final diagnosis that warrants immediate report to the EDCD response team. An intervention could be implemented in the affected area within hours, as opposed to days, potentially preventing additional cases.

The surveillance capacity was enhanced during this season and the hospitals benefitted greatly from the increased resources. In many cases, daily reports came in from the large government hospitals, but for others timely reporting was still a major issue. Staff reported being over-burdened on both sides of the system, both at the ministry of health and in the health facilities, making it difficult to encourage hospital reporting and government follow-up when the reports were not presented. With an at-risk population of

18.5 million people, one potential solution could be to increase or re-route manpower to specifically work on AWD and cholera surveillance at the district level during the monsoon season to ensure all cases are being identified, reported, and responded to. Lessons could also be taken, and trainings created for the less-responsive hospitals, based on those that did report daily even when no cases were seen.

The bar set for a "rapid" response under the CTI approach was to respond to the home within 24 hours of a case presenting to the facility. RRTs were able to perform thorough investigations at the homes of just over half of the confirmed cholera cases, but it took four times as long as planned. Several issues were at play here that can ultimately be traced back to the hospital-based surveillance. First, the vast majority of cases that could not be followed up with household investigation were due to a lack of, or incorrect, contact information for the patient in the medical record. This is often a direct result of an over-burdened and understaffed hospital where the accuracy and completeness of patient information is not a top priority. The EDCD officials' uncertainty around the use of RDTs for case confirmation was a second time-limiting factor. Their preference to wait to call a case confirmed (and thus initiate the household intervention) until the case had been confirmed by culture led to major delays in response. To alleviate this hesitancy, culture was performed in parallel with the RDTs, resulting in a sensitivity and specificity of 90% and 70%, respectively. These numbers support a system in which a response can be initiated by RDT result, and culture can still be used at the national level as gold standard confirmation. Especially in cases where an outbreak has already been detected and confirmed by culture, RDTs are a very efficient surveillance tool. Lastly, it took as

many as six days to respond after culture confirmation. There were only two central-level RRTs devoted to cholera response during the CTI implementation and as the outbreak progressed and daily case count increased, it was more difficult to keep up with household investigations. Manpower issues are a common constraint in Nepal, and one that will need some serious commitment by the government to solve sustainably.

There is no real mechanism for initiating rapid interventions in Nepal's health system. Prior to implementing WASH interventions, planning meetings needed to be held at the district level, even when the same intervention had already been carried out in another area of the same district weeks earlier. On average it took teams nine days to agree upon a location to perform an intervention and obtain the necessary approval to carry it out. While ensuring a quality response is important, it is clear that there is a great need for standard interventions to be agreed upon and planning meetings and trainings to be held prior to the cholera season.

Another major issue with the WASH response was targeting of the intervention. While the program was designed to target those households immediately surrounding a case, interventions were planned and implemented more broadly. The intention was to reach more people at risk, but the result was low coverage of the intervention. In addition to needing a more rapid response, these results seem to highlight the need to narrow the population target for the interventions. However, the interventions themselves did appear to have a positive impact on knowledge in those that received them. One key exception was messaging on cholera vaccination. The intention of the CTI program was to deliver

WASH alongside vaccine to maximize protection. Most survey respondents could name prevention methods for cholera, but very few named vaccination as one of them. This is a major gap in the education provided in these interventions that would need to be filled if the program is to be fully implemented, and succeed, in the future.

A key element of the CTI response was the monitoring of water sources. Nearly all sources were contaminated beyond levels safe for human consumption, however only three water sources were found to be positive for cholera during the household investigations. This sheds light on the state of the water system in Nepal, and the incredible vulnerability of the nation's poor. It is no surprise that improvements are needed in the water and sanitation infrastructure around the country, and steps are being taken, especially in light of this new data. This can serve as a reminder of how interventions such as WASH and OCV can be leveraged to prevent morbidity and mortality while those improvements are made.

The inability to obtain OCV within the program period was a significant obstacle, but it led to a discussion of the need for a small national vaccine stockpile. Without adequate knowledge of disease burden on which to base a pre-emptive vaccination campaign, the proposed reactive strategy provides an efficient alternative. A small stockpile would allow the ministry of health to respond quickly to seasonal outbreaks, but would also provide a safety net in the event of a large outbreak while more resources are being requested and obtained.

2.5.1 Limitations

This is an analysis of feasibility, and there was no attempt to determine the effectiveness of the CTI approach. Measuring effectiveness would require randomization to non-intervention groups and careful consideration of the ethical concerns, but may be considered in the future. Timeliness and practicality were the main criteria used to determine feasibility, however, cost is also a large determinant of feasibility that was not considered in this analysis.

Another potential limitation was the six to eight month lag between the implementation of the WASH interventions and conducting the household survey. Despite the implications for recall and therefore the reliability of the coverage estimate, the results of the knowledge portion of the survey are highly informative. Whether or not the family received or remembered the intervention itself, the results reveal the proportion of the population that has the knowledge necessary to protect themselves and their family from cholera. It has also been argued that surveys are inadequate for collecting data on the personal issues targeted here, such as hand-washing, food hygiene, and proper sanitation practices, since rates of such behaviors are often overestimated.[89]

2.6 Conclusion

The CTI shows promise as a strategy to unify effective cholera control procedures. We understood that this approach would represent a major change in the current procedures for cholera management, since case management, laboratory assays, and public health response are not generally tightly coordinated. The clinician would need to identify the

case quickly and arrange for a rapid test to be carried out. The technician carrying out the test would quickly notify the EDCD of the positive case, and the CTI-RRT could quickly (within 48 hours of the case coming for treatment) visit the neighborhood and implement within this neighborhood an integrated intervention package including WASH, health education, community mobilization and vaccination. Upon its first implementation this timeline has been extended, but through this evaluation we have shed light on the current weaknesses in the cholera surveillance system and identified concrete areas for improvement.

The CTI was successful in raising awareness and engaging stakeholders in both government and private sectors. While the RRT were able to investigate cases at the household within 48 hours of the positive culture result, the analysis identified several constraints that limited a truly rapid response able to target the immediate neighborhood of the case. Using the information from this first year, we identified pathways that will be useful for future implementation of CTI. Issues with the response were extensively discussed post-monsoon season and solutions were integrated into the country's first national cholera control plan. Armed with this experience, increased awareness, available doses of vaccine, and a government and stakeholder-endorsed plan, the CTI approach has the potential to prevent the spread of cholera in the Kathmandu Valley, and eventually around the country.

2.7 Paper One Tables

Table 2.1 CTI feasibility indicators

Indicators	Definition
Time from admission to EDCD notification for a confirmed case	Days (mean and range) from admission to hospital reporting
The percentage of index households found and interventions implemented	Numerator: number of index households found Denominator: total number of cholera cases from the project area detected by the hospital labs
Time from EDCD notification to household investigation	Days (mean and range) from hospital report to household visit
The percentage of index homes and 1st degree neighbors receiving WASH intervention in less than 24 hours after detection of the index case	Numerator: number receiving WASH in under 24 hours Denominator: total number of receiving WASH
The percentage of index homes and 1st degree neighbors receiving WASH intervention in less than 48 hours after detection of the index case	Numerator: number receiving WASH in under 48 hours Denominator: total number of receiving WASH
The percentage of households who report having heard WASH messaging at the household or community level	Numerator: Number of households who received messaging Denominator: Total number of households approached
The percentage of rings vaccinated in less than three days after detection of the index case	Numerator: number of rings vaccinated in less than three days Denominator: total number of rings vaccinated
Number of doses delivered per day during an OCV campaign	Doses (mean and range) delivered each day
The percentage of eligible household members of the index cases who received the single dose of vaccine:	Numerator: number of eligible household members of the index cases who received the dose of vaccine Denominator: total number of household members of the cases
The percentage of eligible neighbors in the defined ring around the index cases who received the single dose of vaccine:	Numerator: number eligible neighbors in the defined ring around the index cases who received the dose of vaccine Denominator: total number of eligible neighbors in the defined ring around the index cases

Table 2.2 Population characteristics

	Patient Population		Survey
	AWD*	Cholera	Respondents
N	2207	169	394
Mean Age (sd)**	35.20 (21.03)	25. 46 (14.03)	38.5 (13.51)
Sex			
Male	975 (44.5%)	79 (46.7%)	151 (38.3%)
Female	1218 (55.5%)	90 (53.3%)	243 (61.7%)
Residence			
Outside Valley	374 (17.5%)	16 (9.6%)	0
Kathmandu Valley	1769 (82.5%)	150 (90.4%)	394 (100%)

*Alkaline peptone water

**Age difference between patient populations is statistically significant ($p<0.001$)

Table 2.3 WASH intervention coverage and messaging

Intervention	N	%
Household Visit by FCHV*		
Respondents who received a visit	65	16.5%
Reported messaging during visit:		
Hand-washing	43	66.2%
Water purification	52	80.0%
Food hygiene	23	35.4%
Personal hygiene	31	47.7%
Sanitation	19	29.2%
Cholera Education	8	12.3%
Reported supplies provided during visit:		
Chlorine tablets	41	63.1%
Water storage bucket	1	1.5%
Soap	4	6.2%
Miking		
Respondents who heard miking	72	18.3%
Reported messaging heard:		
Hand-washing	43	59.7%
Water purification	67	93.1%
Food hygiene	23	31.9%
Cholera Education	20	27.8%
Parasite prevention	2	2.8%
Vitamin A supplementation	1	1.4%
Other WASH Interventions**		
Booth Campaign	15	3.8%
Awareness Rally	13	3.3%
Community Group Meeting	16	4.1%
School Intervention	6	1.5%

N=394 total respondents

* Female community health volunteer

** Water, sanitation, and hygiene

Table 2.4 Knowledge of cholera symptoms, causes, prevention and treatment

	No Intervention		Received WASH Intervention		Total	p-value
	N	%	N	%	%	
General						
Heard of cholera	224	81.5%	109	91.6%	84.5%	0.011
Could identify season	203	90.6%	106	97.2%	92.8%	0.028
Symptoms						
None	24	11.1%	12	11.4%	11.2%	0.922
Diarrhea	168	75.0%	76	69.7%	67.3%	0.307
Vomitting	127	56.7%	63	57.8%	57.1%	0.849
Dehydration	21	9.4%	11	10.1%	9.6%	0.835
Causes						
None	17	7.6%	5	4.6%	6.6%	0.301
Contaminated water	188	83.9%	97	89.0%	85.6%	0.217
Contaminated food	168	75.0%	85	78.0%	76.0%	0.550
Not washing hands	30	13.4%	16	14.7%	13.8%	0.750
Treatment Methods						
None	29	12.9%	9	8.3%	11.4%	0.207
ORS	159	71.0%	86	78.9%	73.6%	0.124
IV fluid	7	3.1%	14	12.8%	6.3%	0.001
Incorrect*	132	58.9%	72	66.1%	61.3%	0.210
Treatment Facilities						
None	72	32.1%	15	13.8%	26.1%	0.000
Governement Hospital	109	71.7%	57	60.6%	67.5%	0.072
Private Hospital	57	37.5%	45	47.9%	41.5%	0.109
Health Post	39	25.7%	35	37.2%	30.1%	0.054
Pharmacy	31	20.4%	10	10.6%	16.7%	0.046
Prevention						
None	15	6.7%	5	4.6%	6.0%	0.447
Safe water	201	89.7%	98	89.9%	89.8%	0.960
Hygienic food	130	58.0%	80	73.4%	63.1%	0.006
Hand-washing	93	41.5%	61	56.0%	46.2%	0.013
Clean latrines	71	31.7%	39	35.8%	33.0%	0.457
Vaccination	3	1.3%	3	2.8%	1.8%	0.363
Prevention Methods Taken by the Household						
None	27	9.8%	4	3.4%	7.9%	0.029
Boiling drinking water	220	80.0%	109	91.6%	83.5%	0.004
Water treatment with chlorine	34	12.4%	39	32.8%	18.5%	0.000
Use of sanitary latrine	89	32.4%	46	38.7%	34.3%	0.227
Hand-washing before preparing meals	115	41.8%	64	53.8%	45.4%	0.029
Hand-washing after defecation	91	33.1%	47	39.5%	35.0%	0.221

Note: frequencies were compared via a chi-square test, red indicates a statistically significant difference in frequency between groups
* Incorrect treatment methods: plain water, liquid food, or no treatment required

2.8 Paper One Figures

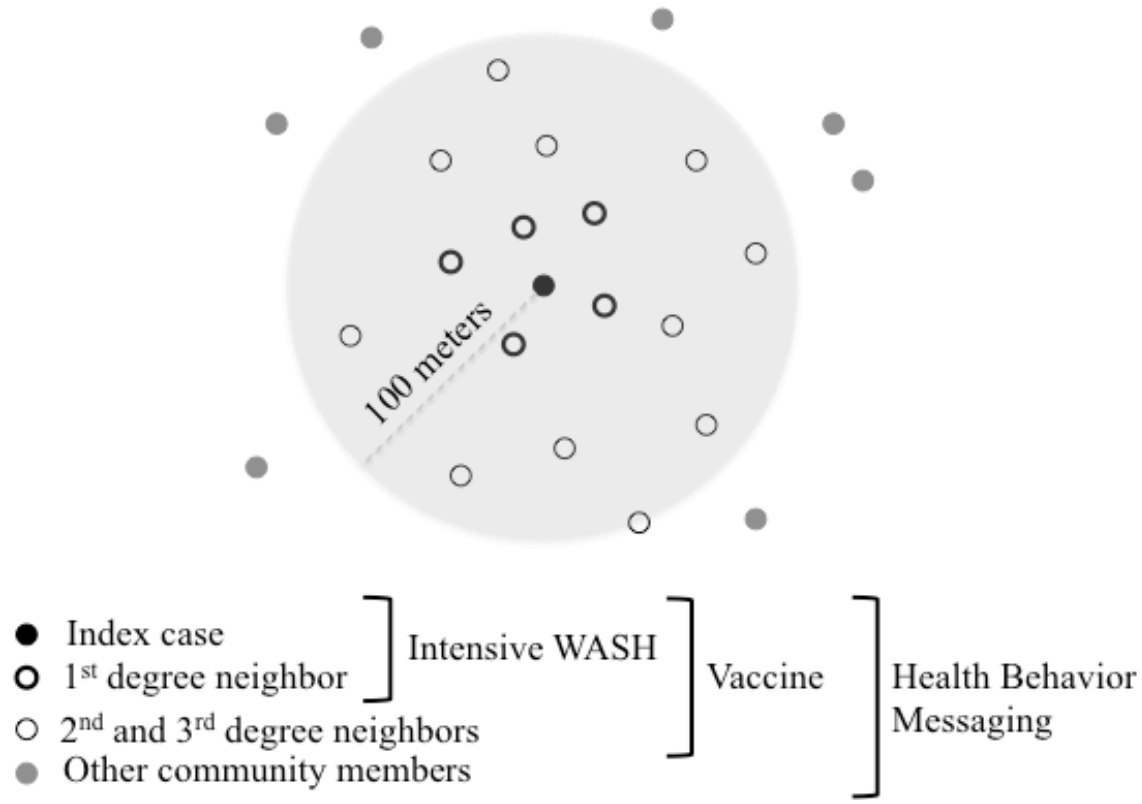

Figure 2.1 CTI ring strategy. A 100m ring is approximately identified around an index case (shaded area). Intervention households are indicated by points, and specific interventions vary by distance from the index household (black point).

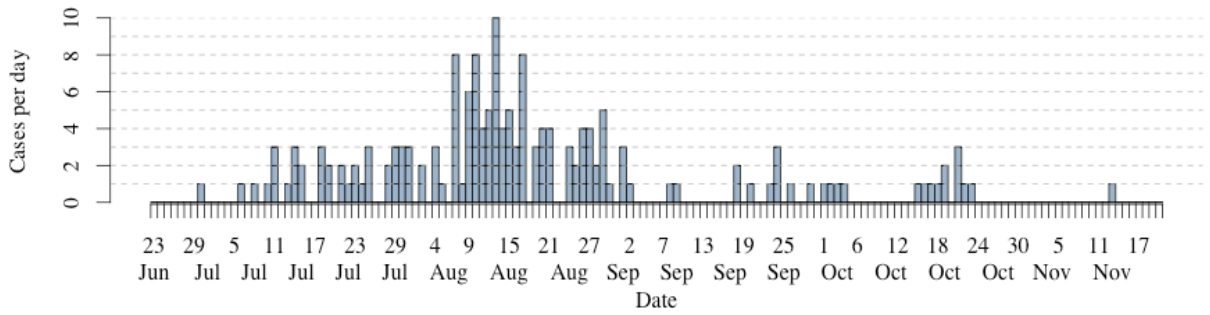

Figure 2.2 Epidemic curve in Kathmandu Valley, Nepal 2016. Confirmed cholera cases shown in bars, and defined as all individuals who are positive for *V. cholerae* by culture (n=169).

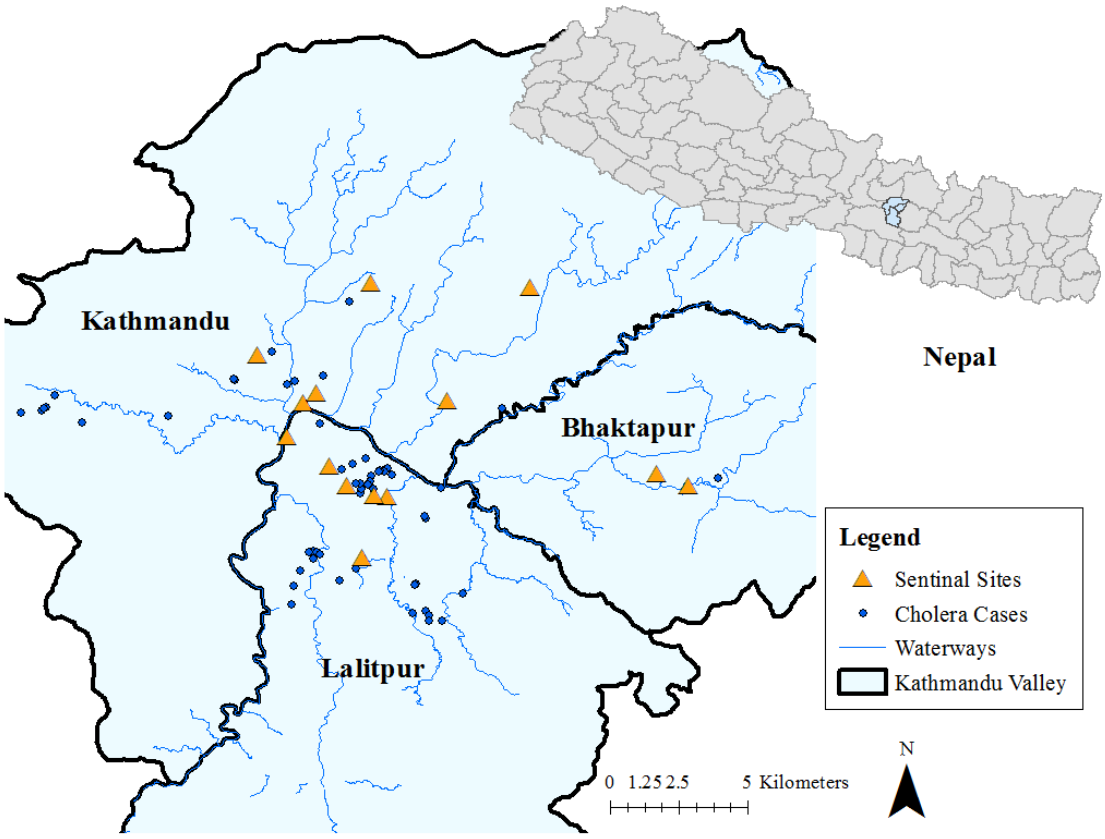

Figure 2.3 Geographic distribution of cholera cases in Kathmandu Valley, 2016.

Points indicate the location of the case. Triangles show the location of hospital sentinel surveillance sites.

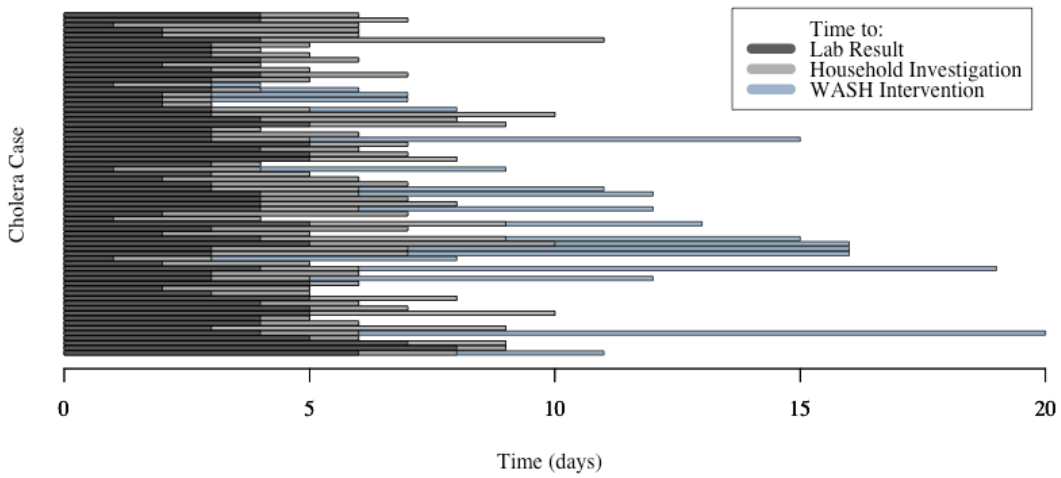

Figure 2.4 CTI surveillance and response performance. Bars indicate the time from hospital admission of the index case to initiation of a WASH intervention in the neighborhood of that case. Data is only shown for cases in which complete date information is available for hospital admission, household investigation, and WASH intervention (n=69).

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CHAPTER THREE

PAPER TWO

GENETIC CHARACTERIZATION OF *VIBRIO CHOLERAE* CLINICAL SAMPLES FROM A 2016 OUTBREAK IN KATHMANDU VALLEY, NEPAL

Mellisa Roskosky[1], Jyoti Acharya[2], Shan Li[3], O. Colin Stine[3], David A. Sack[1]

[1]Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, USA

[2]National Public Health Laboratory, Department of Health Services, Kathmandu, Nepal

[3]University of Maryland School of Medicine, Baltimore, MD, USA

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3.1 Paper Two Abstract

3.1.1 Background

Limited data exists on the molecular characteristics of *Vibrio cholerae* in Nepal. Techniques such as PCR and MLVA can revitalize surveillance and response, not only in terms of time to diagnosis and response, but also for understanding the molecular epidemiology of the disease. We evaluated the genetic diversity of *V. cholerae* clinical samples during a 2016 outbreak of cholera in the Kathmandu Valley.

3.1.2 Methods

Hospital-based surveillance took place at 15 sites within the Kathmandu Valley between June 1st and December 30th 2016. A total of 180 stool samples were collected on filter paper from suspected cholera patients during this period and stored for molecular testing. These samples were tested for cholera via PCR, and the genetic relatedness of positive samples was determined by comparing alleles at five loci containing a variable number of tandem repeats. These results were compared to bacterial culture results obtained during the outbreak, and to previously reported MLVA genotypes from Nepal and elsewhere.

3.1.3 Results

Diagnostic testing via PCR revealed an additional 24 clinical samples positive for *V. cholerae* O1, bringing the total number of confirmed cholera cases during the 2016 outbreak from 169 to 193. MLVA genotyping showed minimal genetic diversity, with only a single clonal complex. The genotypes of the 2016 samples differed from those

previously identified in Nepal. However, some relatedness was seen between the 2016 Nepal samples and large chromosome (three loci) genotypes seen in other countries.

3.1.4 Conclusion

The minimal diversity seen in these clinical samples combined with the shape of the epidemic curve seems to indicate a clonal outbreak consistent with a common source outbreak followed by secondary fecal-oral spread from person to person. Thus, it seems unlikely that there were multiple endemic environmental sources for *V. cholerae* in the Kathmandu Valley in 2016. The results also highlight how PCR can be used as a way to monitor for false negatives obtained via bacterial culture for cholera in the hospital labs as well as identify outbreaks in distant/rural areas.

3.2 Introduction

Cholera is caused by ingestion of the bacterium *Vibrio cholerae* and presents clinically as the rapid onset of acute watery diarrhea, often referred to as rice-water stool, and vomiting. Without treatment the disease can progress rapidly and lead to death from severe dehydration in as little as four hours from onset of symptoms. A total of 51 countries are classified as endemic for cholera, with six of these countries in South East Asia including Nepal and its neighbors China and India.[90] Nepal's Kathmandu Valley routinely experiences cholera outbreaks during the annual monsoon season from June to August.[91-94]

Due to the increased risk for cholera transmission resulting from the 2015 earthquakes in Nepal, an enhanced hospital-based surveillance system was established in the Kathmandu Valley. From June 30th to December 30th 2017, a total of 169 cases of cholera were diagnosed in hospitals around the Kathmandu Valley and confirmed via bacterial culture by the National Public Health Laboratory (NPHL) of the Nepal Ministry of Health.

As part of the enhanced surveillance system, stool samples were collected on filter paper for suspected cholera patients presenting to one of the surveillance sites. These samples can be used for molecular testing and have the potential to drastically shorten the time to confirmation and improve outbreak response and the understanding of cholera epidemiology in the area. In comparison to culture, the use of polymerase chain reaction (PCR) for detection of *V. cholerae* in the stool takes hours as opposed to days. This not only means the case will be confirmed faster, but also that a response can take place

sooner to prevent further infections. PCR also allows increased flexibility in the type of sample required for testing. A fresh stool sample is required for culture, which may be difficult to deliver to a national lab if the hospital is experiencing a high burden or is located in a remote area. A filter paper sample does not need to be refrigerated and is non-biohazardous once dry, avoiding these issues and still allowing for the confirmation to take place.

Outbreak response can also be improved through the use of more advanced molecular techniques, such as multi-locus variable-number tandem-repeat analysis (MLVA) which can differentiate among isolates and determine whether an outbreak over a wide geographic area is due to a single or multiple sources. This information can also be used to link cases and combined with GPS data, to track the spread of the disease in an area.

There has been very limited genetic characterization of cholera in Nepal. While *V. cholerae* isolates collected through routine antimicrobial resistance surveillance have been characterized previously, this is the first instance of longitudinal genetic analysis of a single cholera outbreak in Nepal.[95,96] In order to better prepare for response to cholera outbreaks, it is important to understand the relationships between strains. This work aims to determine the relatedness and potential avenues of transmission of *V. cholerae* strains circulating in the Kathmandu Valley and highlight the importance of molecular characterization of isolates at the national level.

3.3 Methods

3.3.1 Sample Collection

A total of 180 stool samples were collected as part of a hospital-based surveillance system for cholera at 15 sites within the Kathmandu Valley between June 1st and December 30th 2016. A standard case definition was used to differentiate cholera: Acute watery diarrhea (AWD), with or without vomiting, in a patient aged one-year or more. Stool samples from patients meeting the clinical case definition were inoculated into Cary-Blair transport media and sent for culture confirmation and serotyping at the National Public Health Laboratory (NPHL). These stool samples were also spotted on Whatman 903 filter paper cards (Whatman 903 Protein Saver Card, GE Healthcare Ltd., Forest Farm, Cardiff, UK) for later PCR detection and molecular testing.[62]

3.3.2 Bacterial Culture and Antimicrobial Resistance Testing

Cary-Blair specimens were streaked onto thiosulfate citrate bile salt sucrose (TCBS) agar upon arrival at the NPHL and incubated at 37°C for 24 hours. Following incubation, the plates were visually screened for the yellow colonies indicative of *V. cholerae*. After subculture, single colonies were tested for agglutination in anti-O1 and anti-O139 sera to determine the serogroup, and in Ogawa and Inaba anti-sera to determine serotype. Antimicrobial resistance was determined using standard disc diffusion methods for ampicillin, azithromycin, ceftriaxone, ciprofloxacin, cotrimoxazole, nalidixic acid, and tetracycline.[97]

3.3.3 DNA Extraction

Filter paper samples were transported to the US in bulk, after the outbreak in Kathmandu had subsided. DNA was extracted from the dried stool spots similar to published methods.[84] Dried stool spots were cut from the filter paper cards using sterile scissors and then placed into a sterile, labeled tube with 1mL of sterile phosphate buffered saline (PBS). After a 10 minute, room temperature incubation, the sample was centrifuged at 14K RPM for 2 minutes and the supernatant was discarded. Two hundred μL of distilled water was added to the tube, which was then incubated for 8 minutes at 100°C. The sample was then centrifuged for 1 minute at 14K RPM and the supernatant was transferred to a new, sterile tube for storage at -20°C.

3.3.4 Polymerase Chain Reaction

Each sample was confirmed for cholera using a series of multiplex PCR tests. The first identifying samples containing the cholera toxin gene (*ctxA*) and a gene encoding for the cholera outer membrane protein (*ompW*), and the second differentiating O1 from O139.[98-100]

The first reaction was run in a total volume of 25 μL, containing 5 μL of DNA extracted from the dried stool spots, 1x Terra PCR Direct Buffer (with Mg2+, dNTP), 0.5 U Terra PCR Direct Polymerase Mix (Clontech Laboratories, Inc., Mountain View, California, USA), 0.4uM of *ompW* forward and reverse primers, and 0.25uM of *ctxA* forward and reverse primers. PCR conditions were optimized at initial denaturation of 2 minutes at 98°C, followed by 30 cycles each with denaturation at 98°C for 15 seconds, annealing at

64.4°C for 15 seconds, and extension at 68°C for 36 seconds, with a final extension step of 68°C for 7 minutes.[98] The amplified PCR product was analyzed by gel electrophoresis on a 2% agarose gel for 50 minutes and visualized under UV light with ethidium bromide. The amplified products for *ompW* and *ctxA* are 588 and 302 base pairs in length, respectively.

Any specimens that tested positive for *V. cholerae* in the first multiplex PCR reaction were tested to determine if they belonged to serogroup O1 or O139 with a second multiplex PCR reaction. The second reaction was run in a total volume of 25µL, containing 5 µL of DNA extracted from the dried stool spots, 1x Terra PCR Direct Buffer (with Mg2+, dNTP), 0.5 U Terra PCR Direct Polymerase Mix (Clontech Laboratories, Inc., Mountain View, California, USA), 2µM of O1-rfb and O139-rfb forward and reverse primers, and 0.8uM *ctxA* (VCT1, VCT2) forward and reverse primers. PCR conditions were optimized at initial denaturation of 2 minutes at 98°C, followed by 30 cycles each with denaturation at 98°C for 15 seconds, annealing at 57°C for 15 seconds, and extension at 68°C for 29 seconds, with a final extension step of 68°C for 7 minutes.[99] The amplified PCR product was analyzed by gel electrophoresis on a 2% agarose gel for 50 minutes and visualized under UV light with ethidium bromide. The O1-rfb, O139-rfb, and *ctxA* amplicons are 192, 449, and 308 base pairs in length, respectively.

3.3.5 Multi-locus Variable-Number Tandem-Repeat Analysis

Specific primers (**Table 1**) and PCR conditions were used to amplify the sequences of five loci in the cholera genome (VC0147, VC0437, VC1650, VCA0171, and VCA0283) each repeated a variable number of times (between 4 and 31 repeats).[101-103] Three of the loci are found on cholera's large chromosome (VC1047, VC0437, and VC1650) and two on the small chromosome (VCA0171 and VCA0283). The large chromosome loci are typically more stable than those on the small chromosome, although there is some recent evidence that suggests this is not always the case.[101,104,105]

PCR reactions were run in a total volume of 30μL, one for VC0147 and VCA0171 and the second for VCA0283, VC0437 and VC1650. Each reaction contained 1 μL of DNA extracted from the dried stool spots, 10x Buffer (with 50mM Mg2+ and 10mM dNTPs), 5U Taq Polymerase, and 10μM each of forward and reverse primers. PCR conditions were optimized for each set of primers. VC0147 and VCA0171 had an initial denaturation of 5 minutes at 95°C, followed by 35 cycles each with denaturation at 95°C for 1 minute, annealing at 58°C for 45 seconds, and extension at 72°C for 1 minute, with a final extension step of 72°C for 4 minutes. VCA0283, VC0437 and VC1650 had an initial denaturation of 5 minutes at 95°C, followed by 35 cycles each with denaturation at 95°C for 1 minute, annealing at 55°C for 45 seconds, and extension at 72°C for 1 minute, with a final extension step of 72°C for 4 minutes. The amplified PCR products were analyzed by capillary electrophoresis. The number of repeats at each locus was determined by using a 3730xl automatic sequencer and internal lane standards (600 LIZ dye size standard, ThermoFisher Scientific, USA), the Gene Scan program (Applied

Biosystems, Foster City, CA, USA) and the formulas seen in **Table 1**. The five loci were ordered by their location on the chromosome and a five number genotype was assigned based on the number of repeats at each of the loci. The relatedness of each isolate was determined by comparing the genotypes using eBURST software (http://eburst.mlst.net) that predicts the founding genotype and how it branches to subsequent genotypes. Groups of related genotypes, derived from the same ancestor, are called clonal complexes. Genotypes that are genetically related possess identical alleles at four of the five loci.

3.4 Results

A total of 180 stool samples were collected on filter paper at health facilities around the Kathmandu Valley and transported to Baltimore for molecular testing. Of these samples, 118 were simultaneously sent in Cary Blair for culture confirmation on TCBS at the NPHL. Sixty-two of the samples were collected on filter paper only, as they could not be transported within 48 hours of collection, and therefore not confirmed by culture.

The results of the diagnostic testing by culture and PCR can be seen in **Figure 1**. Of the cases for which filter paper was available, bacterial culture confirmed 112 cases of *V. cholerae* O1 Ogawa and 2 cases of *V. cholerae* O1 Inaba. All 114 cases revealed an identical antimicrobial resistance pattern, with sensitivity to ciprofloxacin, azithromycin, tetracycline, and ceftriaxone, and resistance to cotrimoxazole, ampicillin, and nalidixic acid. PCR confirmed that 138 of the 180 total filter paper samples contained *V. cholerae* O1 DNA. Sensitivity and specificity were calculated for bacterial culture using PCR for

the cholera toxin gene (*ctxA*) as the gold standard. Culture was found to be 97.44% sensitive and 100% specific.

When combining cases previously deemed positive by culture (N=169) with those newly identified by PCR (N=24), a total of 193 cholera cases reported to hospital surveillance sites during the surveillance period (**Figure 1**). The revised epidemic curve for the 2016 outbreak can be seen in **Figure 2**. Note that nine of the PCR cases are not included in the epidemic curve, as admissions date information was unavailable for those samples.

Only two of the five loci exhibited genetic variation. VC0147, VC0437, and VC1650 had only a single allele, while VCA0171 had six alleles, and VCA0283 had five alleles (**Figure 3**). These alleles made up a total of eight genotypes from a single clonal complex (**Table 2**). Of the two samples positive for *V. cholerae* O1 Inaba, one was of the founding genotype (9-4-6-12-23) and the other shared a single-locus variant genotype (9-4-6-13-23) with three *V. cholerae* O1 Ogawa cases.

3.5 Discussion

Over the seven-month surveillance period, a total of 193 cholera cases reported to hospital surveillance sites throughout the Kathmandu Valley. Beginning approximately one month into the surveillance period, the outbreak lasted five months, well past the end of Nepal's monsoon season. MLVA genotyping of 138 stool samples, which were positive by PCR for *V. cholerae* O1 revealed minimal diversity, indicating a clonal outbreak. Knowledge of the transmission dynamics of cholera, seen together with the

epidemic curve shown**,** leads to the assumption that this was a common source outbreak followed by secondary person-to-person spread. This is corroborated by the few deviations seen in genotype over time and only one clonal complex, despite cases being seen in multiple districts. Thus, it seems unlikely that there are multiple endemic environmental reservoirs or multiple introductions into Kathmandu Valley. In 2016, Nepal was still struggling with the disruption of already sub-par water and sanitation infrastructure triggered by the 7.8 and 7.3 magnitude earthquakes of 2015. The most likely scenario is one in which the overpopulation of the capital, combined with the favorable climate of monsoon season and less than ideal sanitation conditions, led to the opportunity for local transmission of *V. cholerae*.

None of the eight genotypes reported in this study have been reported previously in Nepal. A 2012 analysis of *V. cholerae* strains from 2007 to 2010 revealed a total of 4 clonal complexes over the time period isolated around the country as part of their antimicrobial resistance surveillance system.[95] The strains previously identified in Kathmandu differ from the 2016 strains. No reports of MLVA genotype have been published since 2012 and MLVA analysis is typically only useful for analysis of isolates collected within a short period of time and small geographic area.[104] However, some evidence suggests that the loci on the large chromosome (VC0147, VC0437, and VC1650) are the most stable and are thought to be the best set for estimating relatedness between strains across large distances.[101,104] When comparing MLVA genotypes of only the three large chromosome loci, the (9-4-6) profile seen in Nepal is related to the same profile seen in Bangladesh (Dhaka: 2002-2005 & Northern Bangladesh: 2010),

Mozambique (2003 & 2009), and Cameroon (2014).[62,101,103,104] Mozambique also reported minimal diversity over a much longer period of time (2002-2012). Cameroon and Bangladesh however, saw greater diversity.

While it may be tempting to conclude that this outbreak in Kathmandu Valley was caused by an introduction from a recent traveller from another endemic area or nation [106], data on cholera in the Kathmandu Valley suggest that outbreaks are a common occurrence especially during the summer monsoon period. Cases began being officially reported as part of Ministry of Health Annual Reports in 2013, and have been documented in the Kathmandu Valley every year since, even prior to the major earthquakes.[81] Cholera has long been considered endemic in Nepal, and while these results do not corroborate an endemic environmental source in the Kathmandu Valley, they certainly do not exclude the possibility. One explanation for this type of yearly, clonal spread could be the presence of a reservoir outside of the Kathmandu Valley, but still within the country of Nepal. Travel to the capital city from distant districts is very common, especially as roads improve and air travel becomes more affordable. Poor surveillance infrastructure in the rural and/or distant districts could explain why cases are annual in the Kathmandu Valley, but perceived to be sporadic in other parts of the country.

Bacterial culture on TCBS for *V. cholerae* is very sensitive and specific, but may miss some cases. This is especially important when dealing with outbreaks in distant or hard to reach areas. The filter paper specimens collected during this outbreak were stored for many months at room temperature prior to carrying out the PCR testing. The samples

were easy to collect, store, and ship since they are not biohazardous, and easy to test in a laboratory that is not necessarily on site. These results demonstrate the utility of PCR to identify additional cases in these instances. This highlights the importance for this technology to be available at the national level for both surveillance and response purposes.

3.5.1 Limitations

This study has contributed to the further understanding of the molecular epidemiology of cholera in the Kathmandu Valley, but with limitations. All 180 samples were from a single outbreak, and therefore tell us very little about the genetic changes in *V. cholerae* over longer periods of time. Previous studies provide some genotype information that can be used for comparison, but the gap in time between studies makes it difficult to connect them. Samples were not consistently collected from all patients meeting the case definition during the outbreak due to several logistical variables. High technician turnover in hospital labs often led to confusion in the study protocol, Many times this resulted in filter paper samples being collected for culture positive samples only, if at all. This was in addition to a high caseload during the height of the outbreak, making the extra collection step more difficult in an already over burdened system. As a result, additional culture negative but PCR positive cases may have been missed. It is important to note that these results pertain only to the Kathmandu Valley, as the enhanced surveillance protocol was not taking place in outside districts.

3.6 Conclusion

This genetic characterization suggests that the most recently recorded outbreak of *V. cholerae* in the Kathmandu Valley resulted from a common ancestor strain that then spread throughout the area, and was most likely aided in transmission by favorable environmental conditions and human hygiene behaviors. This data on strain uniformity has furthered our understanding of the epidemiology of this outbreak, and shed light on the potential usefulness of investing in capacity building for molecular techniques at the national level. The filter paper method of collecting stool specimens for later testing at a laboratory equipped for molecular testing has the potential to reduce under-reporting in documented outbreaks and increase detection of outbreaks in remote areas. This is true not only in Nepal but also in any country with hard to reach areas that lack access to microbiology laboratories. Without a functional, comprehensive surveillance system the true burden of cholera in Nepal, and triggers for its emergence and transmission around the country, will remain undefined.

3.7 Paper Two Tables

Table 3.1 Primers and formulas for *V. cholerae* MLVA

Primer Name	Sequence[a-b]	Range[c]	Formula[d]
VC0147-F	TTGTCATGGCTTGGATTTGG	186–224	(x-150)/6
VC0147-R	TET-ACGTGCAGGTTCAACCGTG		
VC0437-F	CGTTAGCATCGAAACTGCTG	265–301	(x-245)/6
VC0437-R	TET-GTTGCCGCCATCACCAGCTTG		
VC1650-F	CTACCAAGCGGCGGTTAAGCTG	370–440	(x-306)/9
VC1650-R	TET-CCGCTAACTGAGTGACCGC		
VC0171-F	GCTGAAGCCTTTCGCGATCC	316–442	(x-265)/6
VC0171-R	FAM-AGGCGCCTGATGACGAATCC		
VC0283-F	AGCCTCCTCAGAAGTTGAG	118–244	(x-95)/6
VC0283-R	FAM-GGAGGTAGCTACGAATTCTAC		

[a]Tet, 6-tetamidite (Green)

[b]Fam, 6-carboxyfluorescein (Blue)

[c]Expected fragment size range

[d]x = size of the fragment

Table 3.2 MLVA Profile of *V. cholerae* O1 strains collected in Kathmandu Valley, Nepal

Year of Isolation	Serotype	MLVA Profile				
		VC0147	VC04367	VC1650	VCA0171	VCA0283
2007	Inaba	9	3	6	19	16
2007	Inaba	9	3	6	19	17
2007	Inaba	9	3	6	20	16
2007	Ogawa	10	7	6	14	16
2007	Ogawa	10	7	6	14	15
2007	Ogawa	10	7	6	15	15
2007	Ogawa	10	7	6	15	16
2007	Ogawa	10	7	6	13	15
2008	Ogawa	9	3	6	19	17
2008	Ogawa	9	3	6	19	19
2008	Ogawa	9	3	6	19	18
2008	Ogawa	9	3	6	20	17
2010	Ogawa	8	3	6	14	19
2016	Ogawa	9	4	6	12	23
2016	Ogawa	9	4	6	12	21
2016	Ogawa	9	4	6	12	23
2016	Ogawa	9	4	6	11	23
2016	Ogawa	9	4	6	13	23
2016	Ogawa	9	4	6	15	23
2016	Ogawa	9	4	6	15	21
2016	Ogawa	9	4	6	15	22
2016	Inaba	9	4	6	12	23
2016	Inaba	9	4	6	13	23

3.8 Paper Two Figures

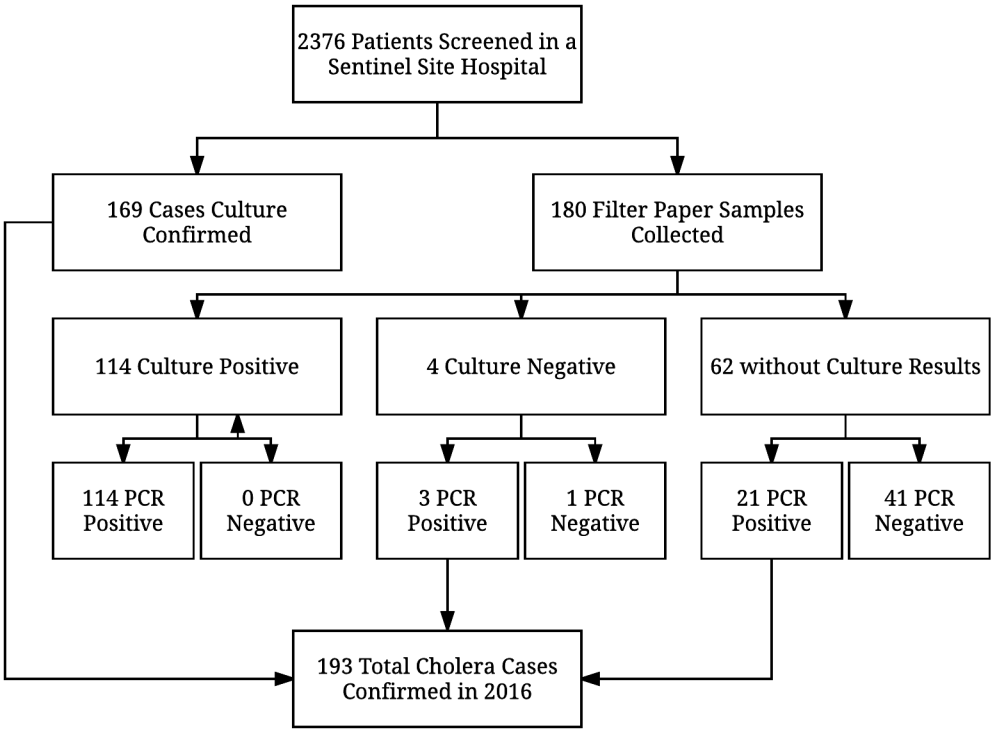

Figure 3.1 Diagnostic testing for cholera by bacterial culture and PCR. A total of 169 cholera cases were confirmed during the 2016 outbreak in Kathmandu Valley. 180 samples were collected on filter paper and tested several months post-outbreak via PCR, resulting in an additional 24 confirmed cholera cases.

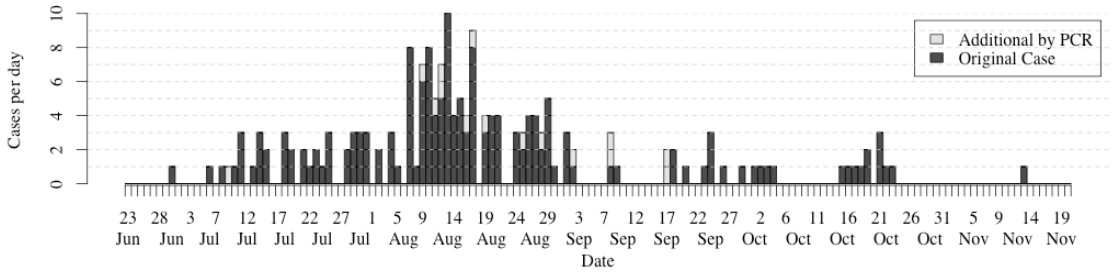

Figure 3.2 Cholera cases per day between June 30th and November 13th 2016. Each box represents a single case. Dark boxes represent cases originally identified by bacterial culture (n=169). White boxes represent additional cases detected via PCR of stool samples collected on filter paper (n=15). Note: Nine samples did not have admissions date and therefore were not included in the above epidemic curve.

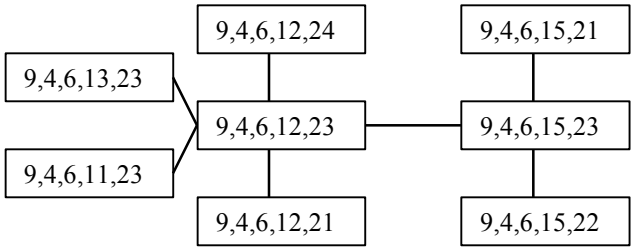

Figure 3.3 MLVA genotypes. A single MLVA clonal complex responsible for a 2016 outbreak of cholera in the Kathmandu Valley. Boxes represent single genotypes (n=8) and lines represent a single allelic change from that genotype.

3.9 Paper Two References

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CHAPTER FOUR

PAPER THREE

SPATIAL CLUSTERING OF CHOLERA CASES IN THE KATHMANDU VALLEY: IMPLICATIONS FOR A RING-VACCINATION STRATEGY

Mellisa Roskosky[1], Mohammad Ali[1], Shyam Raj Upreti[2], David Sack[1]

[1] Johns Hopkins University, Baltimore, MD, USA

[2] Group for Technical Assistance, Kathmandu, Nepal

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4.1 Paper Three Abstract

4.1.1 Background

Despite the availability of effective tools for controlling cholera, it has remained a challenging task; millions are still being affected each year with thousands of deaths. In some cases, a cholera vaccine is being used to control the outbreaks targeting high-risk populations without complete knowledge of their risk status, requiring millions of doses. A ring vaccination strategy targeting the population living around the case was found to be useful in eradicating smallpox and lately in controlling the Ebola outbreak in Guinea. However, such a vaccination strategy has not been evaluated for its ability to stop the transmission of cholera. In mid-2016, a cholera outbreak occurred in Kathmandu Valley, Nepal. This study aims to determine if a reactive, ring vaccination strategy would have been useful in preventing cholera transmission during that outbreak.

4.1.2 Methods

Data on cholera cases were collected as part of hospital-based surveillance for cholera in the Kathmandu Valley from June 30th to November 30th 2016. Cases were confirmed by bacterial culture and/or polymerase chain reaction testing. Subsequent household visits were made to obtain GPS coordinates of the case households. Geographic clusters of cases were visually determined based on potential transmission patterns in space and time, and tested for clustering using Ripley's K and L functions. The cluster size was determined based on the distribution of cases around the index case and reasoning that transmission of cholera would have been stopped if cases occurring within 14 days of the

index case had been vaccinated. The underlying population within each ring was estimated based on 2011 census data.

4.1.3 Results

A total of 193 cholera cases were reported during the surveillance period, of which GPS coordinates of 69 cases were captured. The peak of the outbreak occurred 7 to 10 weeks after the onset. Six geographic clusters were determined, all of which showed significant clustering of cases. Approximately 85% (52/61) of the cases within a cluster occurred more than seven days after the index case. Median ring size was 1km with a population of 14,000 people.

4.1.4 Conclusions

Cholera cases during the outbreak were clustered in space, and the majority of cases occurred over a week after the initial cases in the cluster, allowing for an opportunity to prevent transmission of the disease through use of the vaccine soon after the initial case was identified. This would require only 14,000 doses per cluster when using a single dose strategy. A ring vaccination strategy may be especially useful for large urban areas with recurrent seasonal outbreaks, but where the specific locations for such outbreaks are not predictable.

4.2 Introduction

Cholera remains a significant public health problem in several countries around South East Asia.[90] Six countries in the region are considered endemic for cholera, including Nepal, with an estimated 485 million people at risk. Out of the 818,514 cases and 24,509 deaths estimated to occur annually due to cholera in the region, Nepal accounts for an estimated 30,379 and 911 respectively. These estimates are primarily based on the proportion of people in Nepal without access to improved water (11%) and sanitation (69%) facilities.[90] Cases have been reported sporadically in multiple districts around the country and annually in the Kathmandu Valley over the last 5 years.[81]

Environmentally driven by contaminated waterbodies and seasonal monsoon rains, the disease is subject to secondary spread through fecal-oral transmission.[107] While the primary transmission is responsible for endemicity, it is this person-to-person spread that determines the magnitude of the outbreak.[108] Plentiful data exist in support of cholera's spatial and temporal associations.[77,78,109-112] The closer a person lives to a cholera case, the higher the risk of contracting the disease by that person. Thus, cholera cases tend to be spatially clustered. This observation has led to recent research into the potential for controlling outbreaks via a ring vaccination strategy with a single dose of oral cholera vaccine (OCV). OCV has been shown to be effective in the prevention and control of cholera outbreaks as well as endemic settings.[70] Two-doses of OCV provides over 80% protection during the first 6 months after vaccination. While a single-dose strategy lowers the effectiveness, it has still been shown to provide significant short-term protection and potentially save more lives than the two-dose regimen when the

supply is limited.[71] The evidence suggests that vaccinating a buffer of individuals around an index case, when implemented immediately upon case confirmation, could ultimately halt secondary transmission and prevent the disease from spreading to new areas.[71,77]

Nepal is known to be endemic for cholera, but poor surveillance makes narrowing the target population through hotspot identification problematic. Despite these limitations, the endemic nature within Nepal and the explosive, epidemic potential of the disease given the plethora of risk factors existent in the Kathmandu Valley makes cholera a public health priority.

An outbreak of cholera occurred in the Kathmandu Valley during the summer monsoon months of 2016. This study aims to determine if a reactive, ring vaccination strategy could have been successful in preventing cases once the outbreak began. This determination begins by exploring the transmission patterns of cholera in space and time during the outbreak, with a particular focus on the spatial clustering of the cases.

4.3 Methods

4.3.1 The Study Area and Population

The study was conducted in Nepal's Kathmandu Valley comprising three districts: Kathmandu, Lalitpur, and Bhaktapur. It is the most developed area of Nepal, and is surrounded by four mountain ranges (**Figure 1**). Outbreaks of cholera in Kathmandu Valley have historically occurred during the summer monsoon rains from June to

August.[91-94,113] As per the 2011 census, there were 119 wards in these three districts with a population of about 2.8 million.[114]

4.3.2 The Cholera Data

Data on cholera cases were collected as part of hospital-based surveillance for cholera in the Kathmandu Valley from June 30th to November 30th 2016. A line listing including hospital admission date was maintained for all suspected cholera cases from the 14 chosen hospitals. Fecal specimens from the suspected cases were sent from the hospitals to the National Public Health Laboratory of the Ministry of Health Nepal, who then notified the Ministry's Epidemiology and Disease Control division (EDCD) of any culture confirmed cases. A rapid response team then attempted to obtain permission for a visit to the home of the index case to collect additional information on the case.

4.3.3 The Geographic Data

The geographic data of administrative units (district and ward boundaries) and rivers were obtained from the Ministry of Health, Nepal. As the part of the household investigation, GPS locations of the case households were obtained using a mobile application (KoBo Toolbox, Cambridge, MA, USA) setting World Geodetic System (WGS) 1984. The GPS surveyor was trained to utilize a standard length of time (~10 minutes) to take the GPS readings. The time frame was chosen to assure sufficient time for the GPS receiver to obtain required satellite signals to increase positional accuracy of each reading. Receivers were held static and barrier-free while getting the readings. The GPS readings were then plotted on the ward map and on Google Earth imagery to ensure

accuracy of the data. In addition to case household locations, the locations of the health centers performing surveillance in the study area were collected using a similar technique.

To view and display the landscape of the study area, satellite imagery data of the study area was extracted from Google Earth Pro, which was then georeferenced in the same WGS-1984 projection so that the imagery could be superimposed on to the digital map of the study area.

4.3.4 Spatial Analysis

The geographic clusters of the cholera outbreak were defined within the study area using visual analysis. The outbreak's index case (the 1st case inside the study area) along with the index case in each cluster (1st case within the cluster) were identified by hospital admission date. Linear distances (meters) and times (days) of the secondary cases were calculated in relation to the location and date of presentation of the index case in a cluster. Before calculating linear distance, the geographic data was transformed from WGS-1984 projection to Universal Transverse Mercator Zone-45 North projection. The distance and time of the index case of each cluster from that of the outbreak index case was also calculated. Cases were plotted based on distance and time in order to postulate transmission dynamics within a cluster.

Spatial clustering of the cases was assessed using Ripley's K-function for the cholera case household locations. The K-function estimates the expected number of additional

cases within a range of distances of other cases. To account for the effects of complete spatial randomness, the L-function, which is a variance stabilized transformation of the K-function, was also plotted. The estimates were plotted as a function of distance along with a Monte Carlo assessment of complete spatial randomness to account for uncertainty. Both K and L function analyses were performed using "spatstat" implemented in the R Statistical software, and mapping was done using ArcGIS 10.5.1 (Esri Inc).

The maximum distance from the index case to a subsequent case was recorded for each cluster. This was done for cases occurring within 14 days of the index, reasoning that further spread of transmission would have been stopped covering the cases occurring within 14 days of the index case. Circular rings around each cholera index case were drawn using the median distance of the clusters. The underlying population within each ring was estimated based on the area of the ring overlapped with the area of the ward. Note that the ward is the lowest administrative unit of the Ministry of Federal Affairs and Local Development in Nepal. The ward population was based on the Nepal's 2011 National Census. The following equation was used to calculate the population for each ring (p_j):

$$p_j = \sum_{i=1}^{n} w_i \times \frac{r_j}{a_i}$$

p_j is the population in ring j, w_i is the population in ward i, r_j is the ring j area overlapped with ward i area, and a_i is the area of ward i. The calculations were performed using R Statistical software.

4.4 Results

During the outbreak a total of 193 cases of *Vibrio cholerae* O1 were reported to the Ministry of Health in the Kathmandu Valley through the hospital-based surveillance system. The epidemic curve shows the peak period of the outbreak was 7 to 10 weeks from the date of the initial case of the outbreak (**Figure 2**). Out of 193 cases, the GPS coordinates of the case households were available for 78 households of which 9 locations were outside the study area, leaving 69 cases for the spatial analysis (**Figure 3**).

The spatial distribution of those 69 cases was plotted on a map that clearly shows spatial patterning of the cases (**Figure 4**). Using visual analysis of the spatial distribution of the cases and transmission patterns in space and time (**Figure 5**), six geographic clusters were defined. Global and local (within each of the clusters) clustering of the cases was supported by both the Ripley's K and L functions (**Figures 6A – B** for global clustering and **Figure S1A – B** for local clustering of the cases). In the local cluster analysis, two cases were excluded for which the locations were deemed outliers, as they did not fall within any of the six clusters.

An average of 11 cases were observed in a cluster, with a median cluster duration of 38 days (**Table 1**). Median of the average distance of the subsequent cases in each cluster from the initial case was 0.79 km (Table 1). 85% (52/61) of cases within a cluster occurred more than seven days after an index case reported to the hospital (**Figure 7**), and 44% (27/61) were within 14 days of the presentation. The median of the maximum distance of a subsequent case within 14 days of the initial case in the cluster was 1 km

(**Table 2**). Population sizes ranged from 9,917 people in cluster 2 to over 65,000 in cluster 3 with a median of 14,000 within 1 kilometer of the index case of a cluster (**Table 2)**.

4.5 Discussion

The results of our study suggest that although the outbreak continued for approximately 4 months, the peak was of short duration (4 weeks) as is found in many other situations in Nepal.[115-117] It is noticeable that although the second case occurred in the same place within a weeks time and was likely due to person-to-person transmission, the following three cases occurred two weeks after the initial case presentation. Those cases were far (5 km or more) from the initial case, thus, they were considered to have been infected from the local environment and subsequently transmitted the disease to others nearby over time. When looking at the time distribution of the cases, the majority (85%) of the subsequent cases were infected one week after the initial case in a cluster. This suggests that subsequent cases could have been prevented in each of these clusters had a vaccination program been initiated soon after the initial case in that area was identified.

Subsequent cases within 14 days from the initial case in a cluster occurred within 1 km of the cluster index case. This suggests that a ring vaccination considering 1 kilometer around the index case would be an effective strategy in stopping transmission of the disease, assuming immediate action is taken by the Ministry of Health and that OCV is likely to provide immunity within seven days after immunization.[118,119] Such a vaccination strategy would also be highly cost effective to limit cholera transmission in

the densely populated urban areas by reducing the number of doses usually used to combat an outbreak.[77] The Kathmandu Valley has a population of over 2.5 million and while the amount of available vaccine has increased dramatically over the last few years, demand continues to far exceed supply. Even in the event that the vaccine supply was limitless, vaccinating the entire population would remain logistically and financially infeasible.

The rings with the highest populations in this study overlapped with wards of the Kathmandu and Lalitpur Metropolitan Cities, which have a combined population of 1.2 million. Based on the estimated underlying population in the ring, strategies may need to be designed differently for the metropolitan areas where a high population density may lead to high risk and would require a large amount of vaccine to be available. The average household size for urban areas in the central development region, of which Kathmandu Valley is a part, is approximately 4.24.[114] Thus, the Ministry of Health could anticipate visiting approximately 3,300 households (14,000 persons) per ring in clusters to administer approximately 14,000 doses of vaccine with a single dose strategy.

The cholera cases during this outbreak in the Kathmandu Valley exhibited significant spatial clustering, with an average size of 11 cases per cluster. This pattern indicates the outbreak followed the typical pattern of primary infections from a contaminated water source, occurring almost simultaneously in different areas of the Valley and leading to secondary person-to-person propagation in distinct clusters via fecal-oral transmission.[107] So while contaminated water sources from monsoon rains could be

deemed responsible for the cluster index cases, the bulk of the outbreak is likely determined by poor hygiene and sanitation conditions.[107,120] These results have practical importance in terms of the types of interventions that should be used to control cholera in Nepal, as well as how they should be targeted.

The risk for hospitalization due to cholera was highest during weeks 7 to 10 after the onset of the outbreak. Atypical seasonality can also be seen, as cases continue well after the conclusion of the monsoon season. These observations should inform preparation activities and resource allocation over the course of future outbreaks, such as ensuring additional personnel are available for investigations and interventions in the community during the peak period, or that stockpiles of essential supplies are large enough to see response teams through an entire season.

These results indicate that, within a cluster, the majority of cases occurred more than seven days after the cluster index case. However, previous evidence suggests that the first week after any cholera case, prior to when the vaccine is considered to be effective at preventing illness, is a high-risk period for neighbors of that case.[78] This highlights the importance of delivering additional interventions within this vulnerable period. There are strong calls for the use of water, sanitation, and hygiene (WASH) interventions to be coupled with OCV in these settings. As of now, there is little evidence on effective WASH solutions and sustainability of those solutions. A recent study in Dhaka, Bangladesh found that the WASH program did not reduce the number of diarrhea-related hospitalizations even when the program was run by highly experienced WASH

experts.[121] A recent meta-analysis demonstrated the protective effect of prophylactic antibiotics for preventing cholera among exposed household contacts, while other research warns of the risks of resistance over the course of an outbreak.[122-124] More robust research needs to be done to design impactful interventions for this high-risk period. Given that in this study the majority of the cases occurred one week after the initial case, vaccinating people surrounding the case could effectively reduce the spread of cholera in this instance.

4.5.1 Limitations

While this analysis was performed on a detailed dataset, the data are short term and limited to a single epidemic making it difficult to generalize these results to what can be expected in the coming years. It will be important to continue to monitor the transmission dynamics during future outbreaks to better refine control strategies. The analysis is also limited by the nature of the surveillance data being hospital-based, as it is likely that cases were missed within the community. Kathmandu Valley has many health facilities, but few with the capability for bacterial culture which was a criteria for case confirmation. While sentinel site hospitals were chosen in an attempt to cover the entire Kathmandu Valley population, travel to these hospitals would be an impractical distance for many households on the outskirts of the valley. Even for those making it to the hospital sites, not all cases consented to household follow-up. This limited the number of cases with complete spatial data and therefore does not show the full extent of the outbreak or size of the clusters. Finally, case clusters were defined visually, not adjusting

for the underlying population. It is therefore possible that the spatial pattern seen here might not indicate clustering of cases, but clustering of people.

4.6 Conclusion

In this study, the spatiotemporal dynamics of a single cholera outbreak in the Kathmandu Valley were investigated. Cholera cases during the outbreak clustered in time and space, a pattern that presents an opportunity to prevent further spread via ring vaccination. This analysis is a valuable contribution to the evidence in support of the design of a cholera control strategy; however, the success of such an approach requires vaccine to be readily available and immediately deployed upon case confirmation. The effort required for advanced preparation would be repaid by the prevention of both immediate infections and subsequent transmission of cholera to neighboring individuals and areas. It is likely that this strategy would require even fewer doses in a rural area outside the densely populated Kathmandu Valley, though further research is needed to support this hypothesis. Understanding that the spread of cholera in the Kathmandu Valley is predominately driven by secondary transmission highlights the potential for substantial spread of the disease in a disaster situation and the importance of improving water and sanitation conditions throughout the country. As annual surveillance data continues to be collected on cholera in the Kathmandu Valley it will not only allow for validation of these findings, but more in depth analysis can be performed to better understand the "typical" outbreak in terms of size, household risk-factors, seasonality, and identification of hotspots. This type of detailed longitudinal data will allow health officials to further hone the country's preparedness activities.

4.7 Paper Three Tables

Table 4.1 Spatiotemporal characteristics of cholera case clusters

		Time (days)		Distance (km)	
Cluster ID	Number of Cases	Outbreak Onset to Cluster Onset	Cluster Duration	Primary Index to Cluster Index	Cluster Index to Subsequent Cases (avg)
1	13	0	115	0.00	1.96
2	7	52	41	6.32	0.64
3	20	18	40	6.12	0.86
4	6	108	6	7.95	0.05
5	8	14	16	10.39	0.73
6	13	14	37	7.24	1.05
Median	10.50	18.00	38.50	7.24	0.79

Table 4.2 Maximum distance within 14 days of index case and estimated population by cluster

Cluster	Maximum Distance (km)	Estimated Population within 1km
1	0.0003	53,341
2	0.703	9,917
3	1.66	65,134
4	0.076	16,117
5	1.363	10,686
6	1.79	11,891
Median	1.033	14,004

4.8 Paper Three Figures

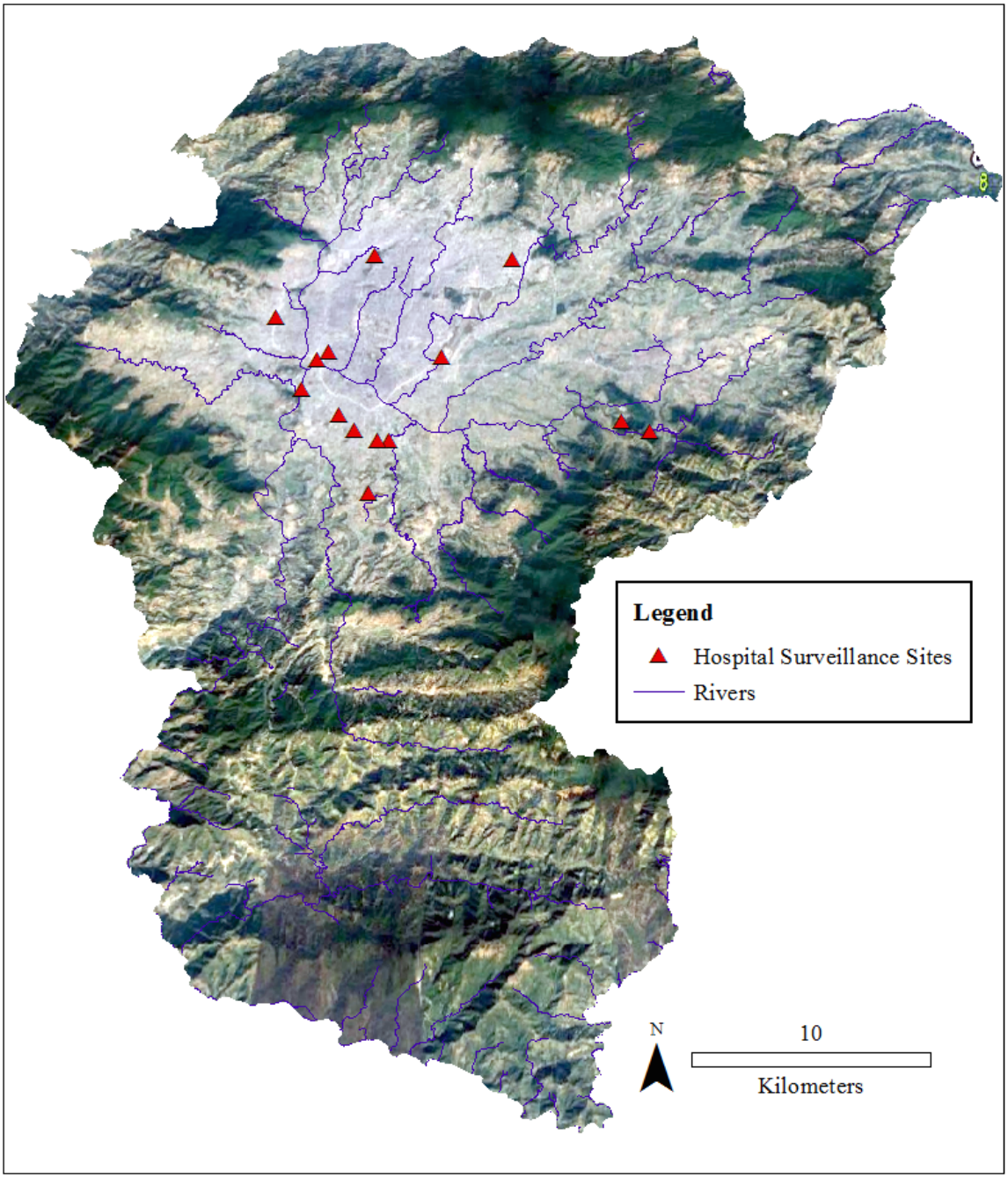


Figure 4.1 Geographic characteristics of the study area and sentinel site hospitals during the 2016 cholera outbreak.

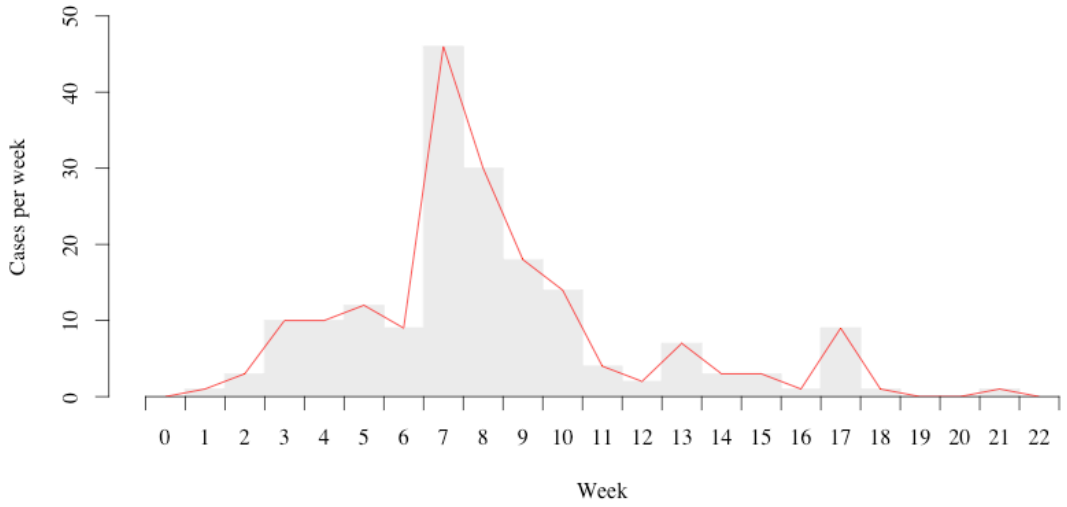


Figure 4.2 Epidemic curve of the cholera outbreak in Kathmandu valley, 2016. This includes all 193 cases in the outbreak

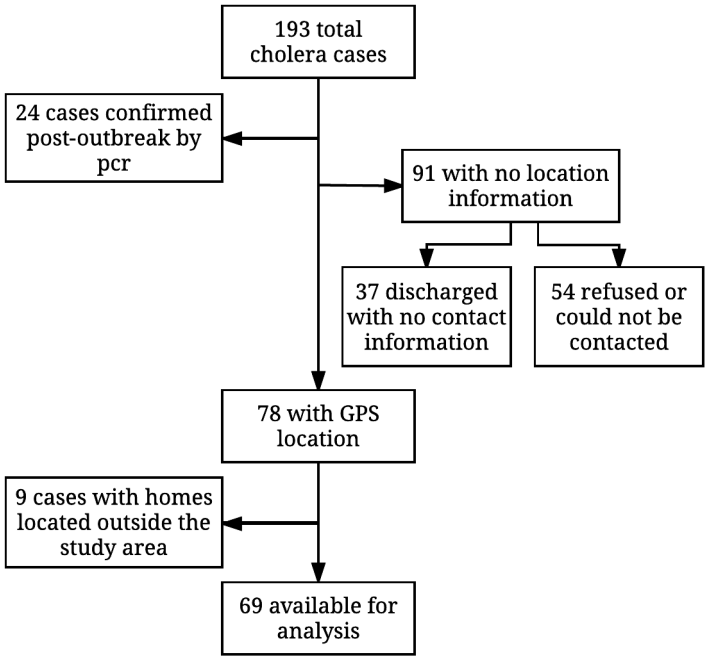


Figure 4.3 Data flow chart. A total of 69 cases were included in the spatial analysis

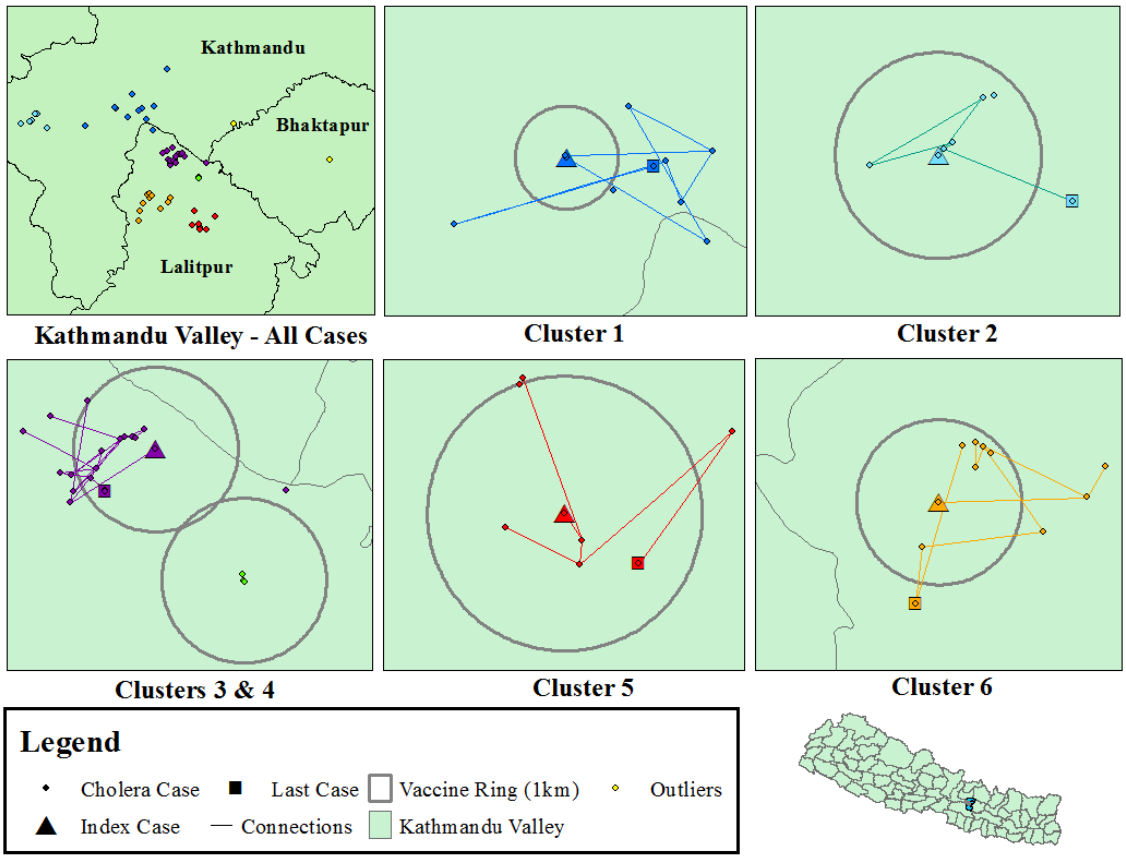


Figure 4.4 Spatial distribution of cholera cases by cluster. The lines indicate connectivity of the cases with respect to time from the initial cases in the cluster. Circles indicate the cases that fall within a 1km radius of the index case.

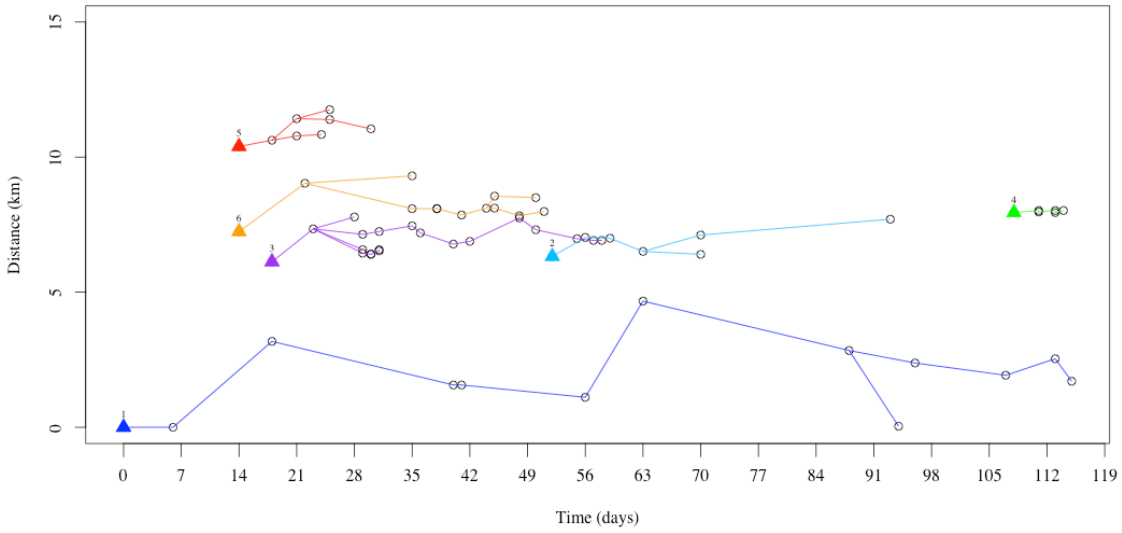


Figure 4.5 Diffusion of cholera in space and time in the different geographic clusters in Kathmandu Valley, 2016. Triangles indicate a cluster index, along with the cluster number. Each point represents a case, with lines indicating potential transmission over time.

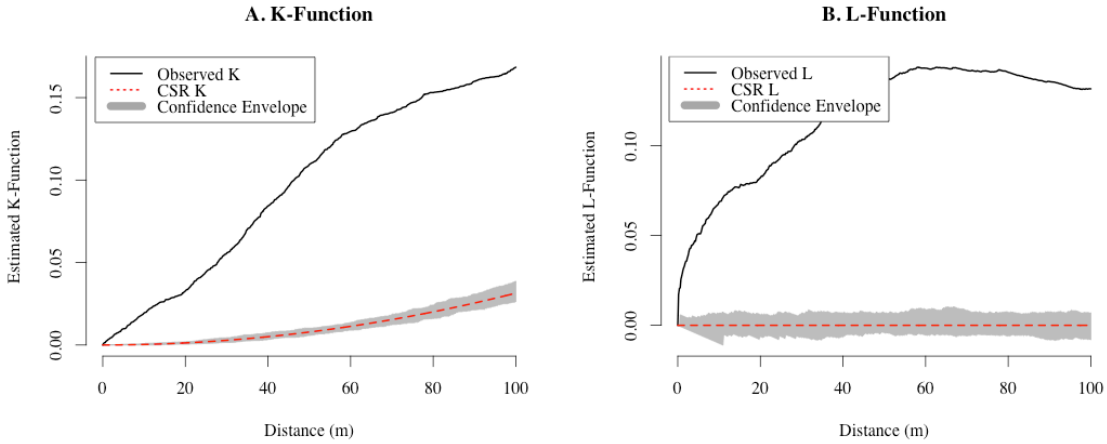


Figure 4.6 K and L functions for global spatial clustering (based on 69 cases). Black lines indicate the degree of clustering that was observed in the data. Red lines indicate what would be expected if cases were completely spatially random. The grey envelope represents the 95% confidence interval.

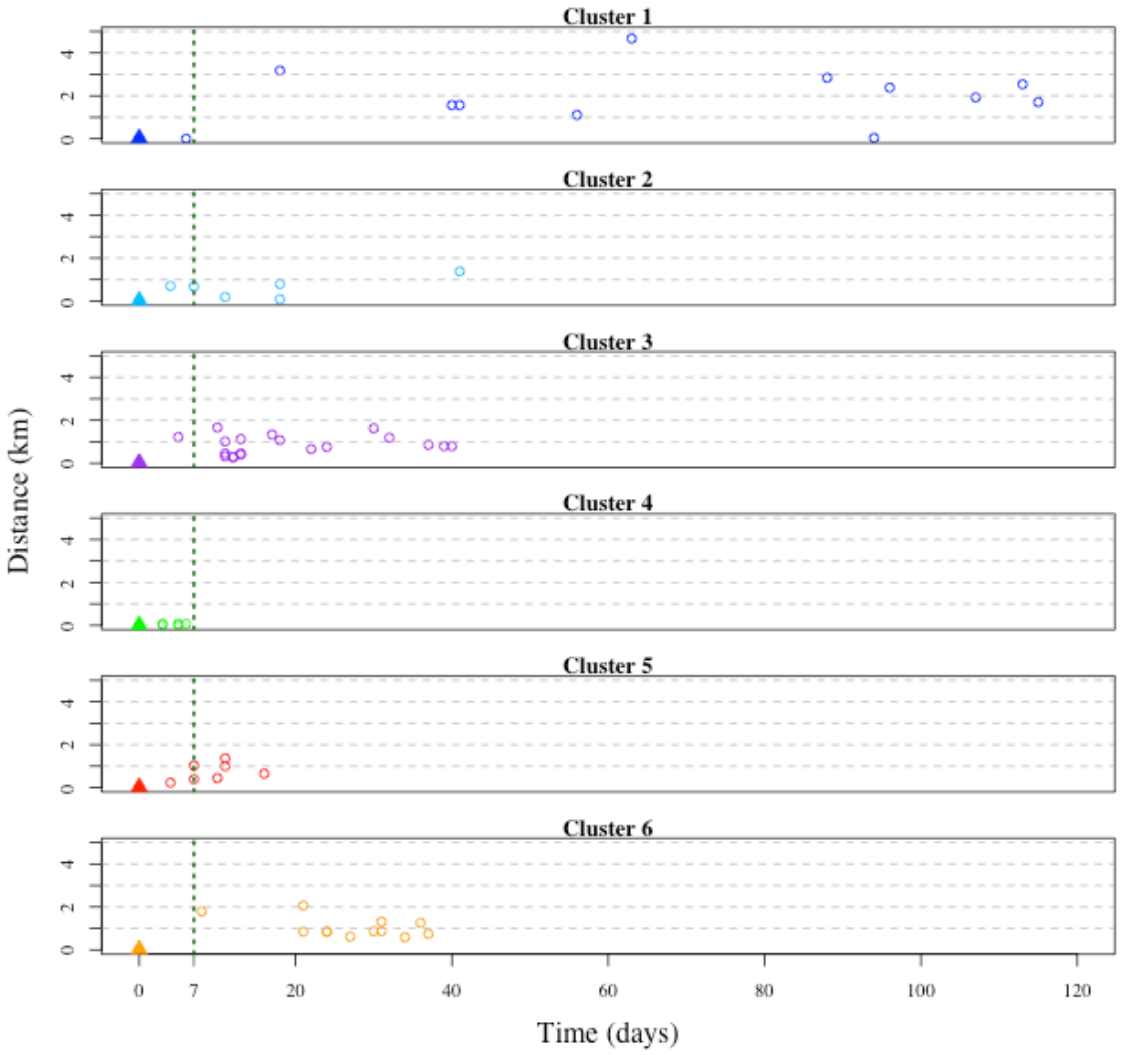
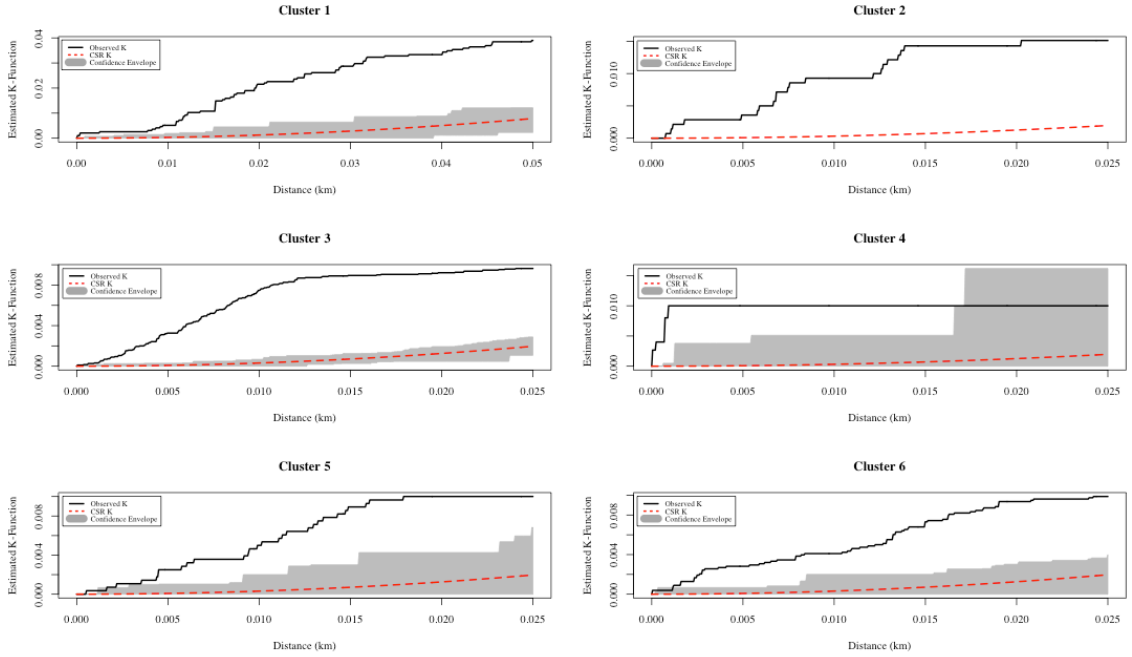


Figure 4.7 Spatial and temporal dynamics of cholera cases by cluster. Distance and time are relative to the cluster index case.

A. K - Function



B. L - Function

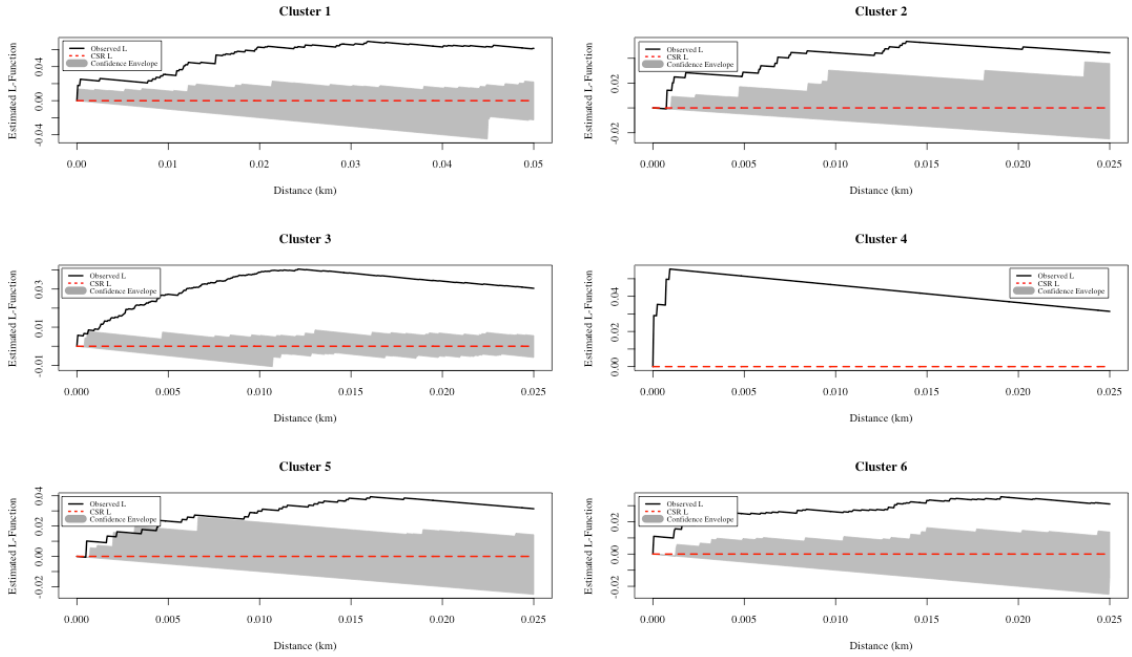


Figure S4.1 K and L functions for local spatial clustering (based on 67 cases grouped into 6 clusters). Two cases were excluded that did not fall into one of the six clusters. Overlap with the confidence envelope can be seen with cluster 5. This is only at very small distances and is likely due to the small number of cases in the cluster.

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CHAPTER FIVE

CONCLUSIONS AND FUTURE DIRECTIONS

The purpose of this dissertation was to design, implement, and evaluate an integrated cholera control strategy in the urban, developing country setting of Nepal's Kathmandu Valley while simultaneously generating data to better understand local cholera epidemiology. Surveillance for cholera in Nepal is inadequate. Despite being considered a cholera endemic nation, with an at-risk population of over 18.5 million, very few resources are devoted to detecting the disease.[90] This creates a two-pronged issue: poor surveillance means few cases are detected and even fewer are confirmed, and lack of data outlining the magnitude of the problem means little assistance is offered by the international community to strengthen surveillance and response activities. These issues are not limited to Nepal and in fact have historically contributed to the limited production of one of public health's key prevention measures, an effective vaccine.[76]

The findings presented here show how a cholera endemic nation can make use of limited resources to prevent the spread of outbreaks and use the associated data to tailor future response efforts, gain insight on the origin of the outbreaks, and better understand disease transmission patterns. The reactive, ring approach outlined in Chapter 2 was carried out in the Kathmandu Valley during the summer monsoon months of 2016. The data collected during that outbreak led to increased knowledge of strain diversity (Chapter 3), transmission dynamics (Chapter 4), and ultimately to the development of the country's first national cholera control plan (Appendix 1).

5.1 Chapter Two

The World Health Organization‘s (WHO) position on oral cholera vaccine (OCV) is that it “complements the other prevention and control measures and should be implemented in relevant settings as part of comprehensive cholera control strategies or while the other activities are being developed.”[125] Those other strategies include water and sanitation improvements, health and hygiene promotion, and strengthened disease surveillance. With little surveillance data to guide targeted mass-vaccination and this integrated approach in mind, the comprehensive, targeted intervention (CTI) for cholera control was born. The CTI combines a strong surveillance network and rapid response teams for household investigations with intensive water sanitation and hygiene (WASH) interventions, behavioral messaging, and vaccination. Chapter 2 demonstrated the feasibility of the CTI approach in the urban, developing country setting of the Kathmandu Valley.

Highlights of the successful piloting included increased awareness surrounding cholera and communication between stakeholders, which allowed rapid response teams to mount a comprehensive response rather than compartmentalized responses at each level of the health system. It also shed light on the barriers to deploying a rapid response, namely the need for an expansion of rapid diagnostic testing at the hospital level for surveillance and response purposes, as well as standard interventions to be agreed upon and planning meetings to be held prior to the cholera season.

As far as specific components of the CTI, water sanitation and hygiene interventions appeared to have a positive impact on knowledge in those that received them despite low coverage. Lack of vaccine during the surveillance period led to the determination that a small stockpile could allow the ministry of health to respond quickly to seasonal outbreaks, but would also provide a safety net in the event of a large outbreak while more resources are being requested and obtained. The project also identified specific unmet needs for effective cholera control, such as rerouting manpower to cholera surveillance at the district level during monsoon season, increasing hospital staff to decrease missed follow-ups, and that improvements are needed in the water and sanitation infrastructure even in the most developed area in the country.

5.2 Chapter Three

As part of the surveillance piece of the CTI approach, stool samples were collected from suspected cholera cases for confirmation of *Vibrio cholerae* by culture and polymerase chain reaction (PCR), and for genetic characterization through multi-locus variable-number tandem-repeat analysis. There has been very limited molecular characterization of cholera in Nepal. Chapter 3 demonstrates the minimal genetic diversity seen among the strains isolated throughout the course of the outbreak. Multiple explanations exist for a clonal outbreak, but the biggest takeaway from the data is that cholera was introduced from a single source and most likely aided in transmission by favorable environmental conditions and human hygiene behaviors.[104,107] That source could be an endemic environmental reservoir within the Kathmandu Valley, or an introduction into the Kathmandu Valley from other areas of Nepal or a foreign traveller. Combined with the

knowledge that cholera occurs every year in the Kathmandu Valley, it is likely that a reservoir exists within the country of Nepal.

Chapter 3 also emphasizes that PCR is the gold standard confirmation method for cholera. This is especially true when attempting to confirm outbreaks in distant or hard-to-reach areas. Bacterial culture on thiosulfate citrate bile salt sucrose is very sensitive and specific, but it can miss cases, and more importantly is not feasible in many areas of the country. Meanwhile, stool specimens can be collected on filter paper, stored for great lengths of time at room temperature, and be tested at a later date for confirmation. These results provide a strong argument for this technology being available at the national level for both surveillance and response purposes.

5.3 Chapter Four

At the heart of the CTI approach is ring vaccination with a single dose of OCV. Evidence suggests that the greatest risk of cholera is in households neighboring an index case and the vaccine has been shown to provide short-term protection with just a single dose.[71] It was designed in acknowledgement of the lack of surveillance data as well as to be respectful of the limited global vaccine supply. Chapter 2 reveals the circumstances surrounding why vaccine was not used during this outbreak, but sufficient data exists to predict if it would have been successful had it been implemented. Chapter 4 confirms that the ring-vaccination approach had the potential to reduce the number of cholera cases during the outbreak.

Analysis of the locations of index case homes suggests that vaccinating individuals within a 1-kilometer ring would prevent many of the subsequent cases. Based on number of cases occurring within each geographic cluster, the average underlying population in the outbreak areas, and availability of vaccine, it was determined that a 1 kilometer ring would be a logistically feasible vaccination strategy. Once inside Kathmandu and Lalitpur Metropolitan Cities, the population totals approximately 1.2 million.[114] In addition to preventing cases, this approach would also be highly cost effective in a densely populated are such as the Kathmandu Valley where vaccinating the entire population would be infeasible.

Chapter 4 also reveals a great deal about the transmission dynamics of cholera in the Kathmandu Valley. Corroborated by the clonal outbreak determination in Chapter 3, this outbreak followed the typical pattern of primary infections from a contaminated water source occurring almost simultaneously in different areas of the Kathmandu Valley, leading to secondary person-to-person spread in distinct spatial clusters via fecal-oral transmission. The primary take-away from this result is that poor hygiene and sanitation conditions are the drivers of the spread of cholera in this area. The risk for cholera was highest 7 to 10 weeks after the outbreak onset, with the majority cases occurring during the first week after an initial index case. Keeping in mind that the vaccine is not considered to be effective until 7 days post vaccination, these findings have important implications for resource allocation, preparation, and the choice of interventions to implement. Understanding how to stockpile resources for a months-long outbreak and

when to expect an increase in need for personnel can mean the difference between effective and sub par responses.

5.4 Policy Implications – Nepal's National Cholera Control Plan

The results of this dissertation have important implications for cholera surveillance and response in Nepal. Chapter 2 highlights the successes of one approach to cholera response but perhaps more importantly some of the key areas for improvement. Understanding weaknesses in the current surveillance and response system are vital to creating an effective control program. Chapters 3 and 4 provide needed evidence for more tailored interventions by revealing molecular and spatial patterns. Translating the evidence into action is the cornerstone of public health practice. As part of the CTI program evaluation a workshop was held with stakeholders from several divisions of the ministry of health, district public health offices, and a number of national and international NGOs to discuss the lessons learned from implementing the program. The result of that meeting was the decision to create a national control plan endorsed by the Ministry of Health to aid in future outbreaks and to outline the responsibilities of each relevant stakeholder in cholera response. The plan was drafted over several months, with input from each stakeholder, and was launched in April 2017. The plan can be seen in Appendix 1. The hope is that this plan will evolve as the understanding of cholera epidemiology in the country advances, and as new tools and techniques become available.

The findings also have implications for policy in other cholera endemic nations, particularly in other countries where poor surveillance leaves the true disease burden unknown. Densely populated urban areas with inadequate water and sanitation facilities exist throughout Asia and Africa and are similarly at high risk for the spread of cholera once introduced. The proposed CTI strategy could be a useful approach for such situations.

5.5 Recommendations for future research

The enhanced surveillance performed during this dissertation work was concentrated in only three of Nepal's 75 districts. Practically no longitudinal cholera incidence data is available outside of the Kathmandu Valley. More work needs to be done to understand how best to expand this surveillance to less developed areas of the country. It is likely that the risk factors differ greatly between districts. While understanding the burden of disease and transmission dynamics in the urban areas is important, it is only a small part of the full picture.

Very little molecular data is available regarding the strains of *V. cholerae* circulating in Nepal year to year. Continued monitoring and MLVA analysis will allow for an understanding of multi-annual patterns of cholera in the country. Is the disease imported, or is there a reservoir, or reservoirs, that exist within the country? These types of data will assist in piecing the strains of *V. cholerae* in Nepal into the overall puzzle of cholera transmission around South Asia and the globe.

While the members of the community that received a WASH intervention during the CTI program appear to have more knowledge of cholera as a result, more research needs to be done on the effectiveness of these interventions. The findings of Chapter 4 demonstrate that a significant portion of the population cannot be protected by vaccination alone, even when rapidly delivered. This seven-day vulnerability window invites additional research into effective WASH and other innovative solutions.

Finally, with the encouraging findings of Chapters 2 and 4, piloting the full CTI ring vaccination strategy in the Kathmandu Valley is the most important next step in evaluating the strategy. A similar ring vaccination approach was attempted in South Sudan, providing more supporting evidence for the approach in general, but in vastly different country contexts.[126]

5.6 Conclusions

In 2017, the WHO's global task force for cholera control outlined a global strategy for reducing cholera deaths by 90% titled, "Ending Cholera: A Global Roadmap to 2030." The document focuses on 47 cholera-affected countries, including Nepal. This dissertation directly addresses two of the strategy's main mechanisms for action: early detection and quick response to contain outbreaks, and targeted multi-sectoral responses to prevent cholera recurrence.[127] The CTI approach is designed to strengthen the ministry of health's ability to detect and confirm cases by utilizing new tools such as rapid diagnostic tests and polymerase chain reaction testing as well as increasing stakeholder awareness and engagement, and is centered around the principle of a rapid

response. It is also an attempt to design a country-specific strategy for integrated cholera control, including WASH interventions, reactive use of OCV, and widespread health behavior messaging coordinated directly with enhanced surveillance. The national cholera control plan produced as part of this work further addresses these mechanisms by focusing on preparedness, stockpiling, and stakeholder coordination. The ultimate goal of the roadmap is to reduce cholera deaths and eventually eliminate cholera, and this will continue to require country-specific research to achieve.

Response to an outbreak is only as good as the surveillance system used to detect it. It is clear that Nepal faces cholera outbreaks on a yearly basis, but the magnitude and geographic extent of the burden is not well quantified. More advocacy at the government level for investment in surveillance training and infrastructure is needed. Interventions such as the CTI have the potential to become even more effective with the right data and resources supporting them. The data generated from these interventions can then be fed back into the design, creating a positive feedback loop where response leads to control and control eventually leads to the elimination of *V. cholerae* as a threat to public health.

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APPENDIX ONE

NATIONAL PREPAREDNESS AND RESPONSE PLAN FOR ACUTE GASTROENTERITIS / CHOLERA OUTBREAKS IN NEPAL

NATIONAL PREPAREDNESS AND RESPONSE PLAN FOR ACUTE GASTROENTERITIS/ CHOLERA OUTBREAKS IN NEPAL

July 2017 to July 2022 AD

GOVERNMENT OF NEPAL
MINISTRY OF HEALTH
DEPARTMENT OF HEALTH SERVICES
EPIDEMIOLOGY AND DISEASE CONTROL DIVISION
TEKU, KATHMANDU

Nepal Goverment
Ministry of Health
Department of Health Services

Tel.: 4252067
4251242
Fax. 4252483

Pachali, Teku
Kathmandu, Nepal

MESSAGE FROM THE DIRECTOR GENERAL

Cholera is an acute intestinal infection caused by ingesting contaminated food or water with the bacterium Vibrio cholera. It quickly leads to severe dehydration and death if untreated. This plan helps the health workers at all levels to clearly understand the case definitions of cholera and manage the outbreaks in a standardized manner with comprehensive approach.

The purpose of this National Preparedness and Response for Acute Gastroenteritis / Cholera in Nepal is, therefore, to enable all the health professionals and partners involved in health and WASH to manage cholera outbreaks in standardized way. The Epidemiology and Disease Control Division initiated to prepare the plan with support from partners in coordination with Task Force for Cholera Control and Steering Committee for Enteric Diseases Control, that would be a step ahead to prevent and control cholera.

Department of Health Services (DoHS) hopes that this plan meets the needs of health workers and the different partners who are participating in Acute Gastroenteritis and cholera cases management.

Dr. Rajendra Pant
Director General
Department of Health Services
Ministry of Health

Government of Nepal
Ministry of Health
Department of Health Services
Teku, Kathmandu

Nepal Goverment
Ministry of Health
Department of Health Services
Epidemiology and Disease Control Division
Teku, Kathmandu

Phone No.: 4261436
Fax No. 4262268
Pachali, Teku
Kathmandu, Nepal

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The development of this National Preparedness and Preparedness Plan for Acute Gastroenteritis/ Cholera Outbreaks in Nepal 2017-2022 has been a long process of consultation and engagement. Governmental and non-governmental stakeholders and partners have dedicated time and resources to ensure a comprehensive plan. The development process included many meetings and detailed input from all partners and stakeholders. This plan is the product of that lengthy process for which the participants at all levels are highly appreciated for their contributions.

The Epidemiology and Disease Control Division, Ministry of Health takes this opportunity to acknowledge the contribution of the following organizations in the development of this National Preparedness and Preparedness Plan for Acute Gastroenteritis / Cholera Outbreaks in Nepal 2017-2022 and many others who may not have been listed.

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Lastly, I would like to thank all relevant organizations and personnel who supported to develop and finalize the plan.

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Dr. Bhim Acharya
Director

TABLE OF CONTENTS

ACRONYMS

AGE	Acute Gastroenteritis
AWD	Acute Watery Diarrhea
CHD	Child Health Division
DFTQC	Department of Food Technology and Quality Control
DPHO	District Public Health Office
DoHS	Department of Health Services
DWSS	Department of Water Supply and Sanitation
EDCD	Epidemiology and Disease Control Division
EWARS	Early Warning and Reporting System
GTA	Group for Technical Assistance
HMIS	Health Management Information System
IEC	Integrated Information, Education, and Communication
IVI	International Vaccine Institute
KVWSMB	Kathmandu Valley Water Supply Management Board
KUKL	Kathmandu Upathyaka Kanepani Limited
LMD	Logistic Management Division
MoH	Ministry of Health
NHEICC	National Health Education, Information and Communication Centre
NPHL	National Public Health Laboratory
OCV	Oral Cholera Vaccination
OPD	Out Patient Department
PCR	Polymerize Chain Reaction
RRT	Rapid Response Team
WSSDO	Water Supply and Sanitation District Office
UNICEF	United Nations Children's Fund
WASH	Water, Sanitation and Hygiene
WHO	World Health Organization

DEFINITIONS

Acute Watery Diarrhea (AWD): The passage of three or more three loose stools per day or more frequent passage than in normal for the individual.

Suspected Cholera: any person aged 3 years or more with moderate or severe dehydration from 3 or more episodes of acute watery diarrhea per day (24 hours), with or without vomiting.

Probable Cholera: any suspected cholera case with a positive RDT or any death in a person 14 years or older resulting from AGE.

Confirmed Cholera: Any suspected or probable cholera case that has *Vibio cholerae* isolated from their stool by bacterial culture or PCR.

Cholera Outbreak (seasonal): A situation where cases of cholera occur in numbers similar to what has been seen in previous years among a specific population

Cholera Outbreak (emergency): A situation where more cases of cholera occur than are expected in a given area, or among specific group of people, over a particular time period (WHO EWARN).

In case of Nepal, 'more than expected' refers to

i) a 50% increase in the number of cases seen in a district as compared to the average over the last 5 years where hospital facilities are available or

ii) a 10% increase in the number of cases seen in a district as compared to the average over the last 5 years where hospital facilities are not available.

Executive Summary

In order to guide the planning and response process for cholera preparedness and control in Nepal, the following strategy has been developed. This document outlines the current cholera situation in Nepal (as of January 2017) and knowledge surrounding interventions that are relevant in the Nepali context. An in-depth SWOT analysis has been included to shed light on the current strengths and weaknesses of the health and donor systems in Nepal, as well as opportunities for program improvement and threats to its success.

The main objective of this plan is to improve the overall health status of Nepal by reducing the incidence of cholera throughout the country. The plan is based on a series of specific objectives: to prevent the spread of cholera, to reduce mortality from cholera, to ensure a coordinated and collaborative response to cholera outbreaks, and to ensure that a rapid response mechanism is in place in order to successfully stop the spread of disease. Each objective is discussed in detail, with several action items included along with when the activities should be performed and who is responsible for their completion. This is intended to serve as both a guide and a source of accountability for the responsible parties.

It is essential to any successful response program to perform routine, annual monitoring and evaluation. The concluding section of this document outlines performance indicators to be used to measure success and areas for improvement. It also details how this evaluation should be conducted and who is responsible for ensuring it is carried out. The results of this evaluation will be a valuable resource for the Steering Committee for Enteric Diseases and the Task Force for Cholera Control in their annual review of this plan, and coordination for the upcoming cholera season.

Annexes are included at the end of the document to assist in systematic reporting, ensuring proper methods, and to provide standardized health education materials for the cholera response and outbreak analysis.

It is assumed that all humanitarian organizations involved in cholera response in Nepal will contribute to the outlined emergency stockpiles and follow this detailed plan. Moreover, It is hoped that this document will serve as more than just a government action plan, but also as a resource at all levels of the cholera response and for all relevant stakeholders.

1. Background

Cholera: The Disease

Cholera is an acute intestinal infection caused by ingestion of food or water contaminated with the bacteria *Vibrio cholerae.*[1] Globally, it is estimated that there are 1.4 to 4.3 million cases, and 28,000 to 142,000 deaths due to cholera every year.[2] Cholera has a direct negative impact on public health and economic productivity, as well as significant indirect costs affecting the health system, social welfare, tourism, trade, investments, etc. The threat of a cholera outbreak is a major public health concern for governments and the international health community, and a key indicator of lack of social development.[3]

Cholera Epidemiology in Nepal

Nepal is endemic for cholera with the potential for large outbreaks. While 93 percent of households in Nepal use an improved source of drinking water and 72 percent of Nepali's live in households with improved sanitation facilities, open defecation is still practiced in many areas.[4] The country is also at high-risk for outbreaks due to a steady increase in urban population density accompanied by an inadequate supply of safe drinking water and improved sanitation. Perhaps most importantly, Nepal faces flooding and landslides during the rainy season every year which often lead to the breakdown of the already fragile water and sanitation infrastructure. All of these complex factors raise the possibility of cholera outbreaks, which may be challenging to prevent and control.

Outbreaks of cholera are reported in different regions of the country every year, causing the location of outbreaks difficult to predict. However, cases are reliably reported within the Kathmandu Valley every year, making it a priority area for cholera control in Nepal. While many of these cases are only clinically diagnosed (**Table 1**), several outbreaks have been laboratory confirmed.

Table 1: Regional Suspected Cholera Data as Reported in the Annual Report of the Department of Health Services

S.N.	Region Name	Cholera Cases							
		2067-68 (2011)		2068-69 (2012)		2069-70 (2013)		2070-71 (2014)	
		n	%	n	%	n	%	n	%
1	Eastern Region	2101	45.9	488	28.0	939	18.6	824	19.8
2	Central Region	825	18.0	431	24.7	2428	48.2	2616	62.7
3	Western Region	443	9.7	302	17.3	447	8.9	205	4.9
4	Mid – Western Region	863	18.9	115	6.6	525	10.4	54	1.3
5	Far – Western Region	345	7.5	408	23.4	703	13.9	471	11.3
	National	4577	100.0	1744	100.0	5042	100.0	4170	100.0

Source: HMIS, DoHS[5]

Recent laboratory confirmed outbreaks are listed below:

- 2009 – Mid/Far Western Region – 1400 clinical and 109 laboratory confirmed cholera cases (NPHL)
- 2010 – Mid /Western Region – 61 laboratory confirmed cases (NPHL)
- 2011 – Kavre District – 1 laboratory confirmed multi-drug resistant case (NPHL)
- 2012 – Western Region and Kathmandu Valley – 35 laboratory confirmed cases (NPHL)
- 2012 – Eastern Region – 4 laboratory confirmed cases (BPKIHS)
- 2012 – Doti and Dailekh Districts – 24 laboratory confirmed cases (NPHL)
- 2013 – Kathmandu Valley – 4 laboratory confirmed cases (1 from NPHL, 2 from Nepal Medical College and 1 from KIST Medical College)
- 2015 – Kathmandu Valley – 80 laboratory confirmed cases (NPHL)
- 2016 – Kathmandu Valley – 169 laboratory confirmed cases (NPHL)

Early Warning and Reporting System (EWARS)

The EWARS was established by the Epidemiology and Disease Control Division of the Department of Health Services in 1997 in order to strengthen the flow of information on vector-borne and other outbreak prone infectious diseases from the district to the national health departments. This surveillance system is hospital-based and is currently operational in 61 out of 82 sentinel sites throughout Nepal. So far, the EWARS mainly focuses on the daily and weekly reporting of number of cases and deaths (including "zero" reports) of six priority diseases, including acute gastroenteritis (AGE) and Cholera. It equally focuses on immediate reporting (to be reported within 24 hours of diagnosis) of even a single suspected case of cholera, as well as five or more cases of AGE from the same geographic location in a one-week period.

EWARS employs two forms of surveillance: indicator-based and event-based. Indicator-based surveillance is the routine reporting of cases of disease, including notifiable disease surveillance, sentinel surveillance and laboratory-based surveillance systems. This routine reporting is commonly health-care facility-based, with reporting on a weekly basis. Event-based surveillance is the organized and rapid capture of information about events that are a potential risk to public health. This information could be rumors or other ad-hoc reports transmitted through both formal (i.e. established routine reporting systems) and informal (i.e. media, health workers and nongovernmental organizations reports) channels as per need.

EWARS offers a complementary system for the detection of a cluster of conditions or unusual events, with a reduction in workload. In the experience of WHO's Surveillance Medical Officers and their counterparts at the District Health Offices, the system has in the past been effective in tracing rumors or investigating potential outbreaks. Rapid Response Teams (RRTs) can be mobilized on short notice to facilitate prompt outbreak response at the Central, Regional and District level, and can also support the local levels for investigation and outbreak control activities.

Scientific Foundations for the Control of Cholera in Nepal

Antibiotic Resistance

Several studies of *V. cholerae* have shown drug resistance to a number of antibiotics. A brief report in 1996 was the first to discuss antimicrobial resistance and noted reduced sensitivity to nalidixic acid, co-trimoxazole, ampicillin, and cephalexin.[6] Almost 12 years later in 2004, cholera isolated during an outbreak in Kavre district was found to be completely resistant to co-trimoxazole, but sensitive to all other antibiotics tested.[7] Another study conducted that year found resistance to nalidixic acid as well.[8] By 2008, an outbreak in Kathmandu (and later in Saptari and Jajarkot) produced isolates that were 100% resistant to furazolidone in addition to co-trimoxazole and nalidixic acid. [9-13] A later study identified this resistance pattern as early as 2005.[14] Resistance to trimethoprim, sulfamethoxazole, and decreased susceptibility to ciprofloxacin was reported in 2010 and to streptomycin in 2012.[15-16] Multi-drug resistant *V. cholerae* has also been identified in Kathmandu's sewer system.[17] The variation in resistance profile results in the need for continuous monitoring to ensure effective drugs are being used when necessary.[16]

Rapid Diagnostic Testing

Effective surveillance is needed to detect cholera cases during the early phases of an outbreak in order to mount the most effective response. High-quality surveillance depends not only on the ability to detect suspected cases of cholera at the health facility level, but also to confirm that those cases are indeed cholera in the laboratory (stopcholera.org). Establishing a high-quality disease surveillance system is not simple and can be both expensive and difficult, especially in the field with limited laboratory capacity, as is the case in most districts in Nepal. Bacterial culture confirmation of *Vibrio cholerae* has been the historical gold standard for cholera confirmation in Nepal, but it requires a well-equipped laboratory with trained laboratory technicians.[18] When this is not available in the outbreak setting, samples must be transported to a reference laboratory which takes time, delaying the response and diminishing effectiveness of interventions. Fortunately, rapid diagnostic tests can be used to diagnose O1 and O139 *V. cholerae* at the point of care without a lab or technician. In comparison to culture, the rapid diagnostic test is about 90% sensitive and 60-70% specific in the field, but the specificity has been shown to increase to over 99% with the addition of an enrichment step.[19-24] This information highlights RDTs as a rapid, low-cost, simplified method for cholera detection in Nepal.

Oral Cholera Vaccination

Oral cholera vaccine (OCV) is an effective intervention for the prevention of cholera, especially when combined with WASH activities.[25] The WHO currently recommends three killed, whole-cell vaccines that are administered orally. Two of these vaccines, Shanchol and Euvichol, have been used in pre-emptive vaccination campaigns in Nepal. These vaccines have been proven safe, and are recommended for adults aged 1 year and over. Typically, two doses are given, two weeks apart which results in 85% efficacy after 6 months in those greater than 1 year of age, 45% after 5 years for those 1-5, and 65% after 5 years for those over 5.[26-27] This plan recommends a two-dose strategy for pre-emptive campaigns. However, recent evidence suggests that during an outbreak situation, a single dose of cholera vaccine during a reactive vaccination campaign is effective at stopping transmission.[28] It has the added benefit of eliminating many of the logistical concerns that often arise from the delivery of a second dose to the same population. For these reasons, a single dose strategy is recommended for reactive OCV campaigns in Nepal.

2. Analysis of Response Capacity (SWOT)

Strengths and Weakness

	Strengths	Weakness
Surveillance	• Cholera is a reportable disease in the Nepal Early Warning and Reporting System • Most cholera cases were tracked down because of early reporting and early signals • Daily situation reports and weekly bulletins prepared and disseminated to higher authorities (PM's office, MoH, WHO, UNICEF)	• Mapping of epidemics has not taken place systematically, making it difficult to create risk maps for the country • Surveillance reporting is incomplete within the EWARS system and not enough sentinel sites are included in the network (unable to detect cases from peripheral levels) • Electronic reporting system not used at all sites • Irregular zero reporting from sentinel hospitals • Incomplete contact information recorded at sentinel hospitals • Not all health facilities are included in the surveillance system
Laboratory Diagnosis	• Newly developed SOPs were consistently used, resulting in adherence to high standard in culture confirmation • Where appropriate decentralize culture confirmation from national to hospital level • Samples from outside Kathmandu were transported to NPHL properly • NPHL is well equipped for the confirmation of cholera via culture	• Long duration of incubation to conduct enriched RDT test (more than 6 hours) • Timely availability of RDTs and supplies for culture confirmation • Sample transport from peripheral to central level for laboratory confirmation delays response • SOPs do not cover how to proceed with testing outside normal working hours as samples are received 24 hours a day • Untrained personnel performing RDT and culturing • TCBS is not routine in outside Kathmandu valley

	Strengths	Weakness
Field Investigation	• Water samples collected from households during investigation • High percentage of cases are followed up at their homes and data collected 2016 • RRTs are in place at the central, regional, and district levels for outbreak investigation and response	• RRTs are not trained to conduct scientific outbreak investigations • Shortage of qualified human resources (e.g. field epidemiologists)No dedicated statisticians at any level • No implementation of food safety monitoring due to low perceived priority and lack of coordination with concerned authorities
Case Management	• Few deaths have been reported • Antibiotics are readily available in hospitals	• Many patients are discharged from hospital early, instead of remaining for fluid replacement • Health personnel are not trained to handle cases of cholera • Patient details are often not fully / properly recorded • Health education before discharge is not practiced systematically • No clear cut SOPs on patient and contact tracing
Water Supply and Sanitation Infrastructure and System	• Regular chlorination of big water supply systems especially KUKL • Use of field water test kits by response team – easy to use • Coordination with water tanker and other private service providers • Water quality monitoring data as a triggering tool for identifying cholera outbreaks • Initiation of directives/ policy for water tankers	• No water system mapping, which hinders attempts to trace sources of outbreaks in the system • Responsibilities for water chlorination during cholera outbreaks was unclear. • Monitoring mechanism is not systematized to ensure regular quality – for microbiology • Lack of coordination on sharing of water quality testing results among WASH and health sector

	Strengths	Weakness
Cholera Vaccination	• Effective vaccines exist • OCV has been efficiently deployed in the field in a preventative manner in Nepal	• Multiple applications to the stockpile have been denied limiting the ability to deploy vaccine • Financial sustainability for procurement • No national plan or policy on use of OCV in preparedness or response to cholera outbreaks
Communication Campaigns and Social Mobilization for Safe WASH Practices	• Optimal prioritization of at risk populations • Timely expansion of interventions to cover larger areas • Mass media campaigns reached beyond target areas • Mobilization of FCHVs & volunteer networks from affected communities	• Lack of preparedness plan of BCC interventions • Limited time for volunteer training on community mobilization and BCC • Unavailability of a dedicated team for monitoring • No funding sources of the government to initiate immediate response • No stock of IEC materials at municipality and district levels
Leadership and coordination Mechanism	• Under the chairmanship of DoHS DG, technical and strategic direction for cholera control were provided by Steering Committee for Enteric Disease Control and Disaster Health Working Group • Most outbreaks are small and localized making them easier to respond to and contain • Good coordination between EDCD, NPHL, DPHO, defense sectors, municipality and external development partners	• Perception that overall case numbers are small, leading to low priority on government health agenda and little donor support • No monitoring and enforcement of food safety regulation for small restaurants and street food vendors • Public health issues were low priority in municipality • Lack of coordination with food vendors • Instituting a coordination mechanism across concerned sectors • No clear national criteria to declare the cholera outbreak

Opportunities and Threats

	Opportunities	Threats
Surveillance	• EWARS system is already in place in all 75 districts • There is room and political will to expand this system • External agencies are ready to help in strengthening existing surveillance system • Health system of the defense sector could be included in EWARS	• Continued poor surveillance making it impossible to accurately determine disease burden in the country (only 50% of sentinel sites are functional) • Manpower restrictions could limit the EDCDs ability to follow-up with hospitals if the system were expanded • Lack of motivation, dedication and will power from staff working in surveillance system • Lack of experts in strengthening surveillance system
Laboratory Diagnosis	• Successful use of the Rapid Diagnostic Test for cholera in the Kathmandu Valley, with the potential for expansion to other areas of the country • Simple training on RDT procedure for health staff working in periphery	• Lack of adequately equipped labs around the country capable of diagnosing cholera on site • No dedicated budget for purchase of RDTs
Field Investigation	• Rapid Response Teams are a built-in resource of the EWARS system and has been expanded during the 2016 cholera season in Kathmandu • Inclusion of Response Team from the defense sector, volunteers and private organizations in emergency situations	• Lack of funding to support the expansion of RRTs may limit their ability to respond to every cholera case • Denial by some patients to participate in household investigation due to lack of knowledge and/or stigma • Loss to follow-up due to movement • Difficulties identifying actual sources of drinking water due to use of multiple sources
Case Management	• Routine monitoring of antibiotic resistance means changes can be made to treatment recommendations during an outbreak if necessary	• Limited supply of needed medical supplies (such as IV fluids) in rural health facilities • Frequent stock outs of medicines and supplies

	Opportunities	Threats
Water Supply and Sanitation Infrastructure and System	• At home, point of use disinfectant products are available in markets and can be a major message of health information campaigns in at-risk areas • Protection of source of drinking water by DWSS • Many households use standard size black water tanks which could make interventions at these households much easier (e.g. correct concentration of chlorine)	• The cost of these materials is not regulated, and could be a major factor in a household decision to use chlorination at home • Limited availability of hand washing stations in the home
Cholera Vaccination	• Increasing availability of OCV in the market, making it easier to apply to the global stockpile	• Global supply of OCV is limited • Due to lack of funds for direct purchase, the government must rely on donations or successful applications to the stockpile
Communication Campaigns and Social Mobilization for Safe WASH Practices	• Strong partnerships with organizations such as UNICEF means many opportunities to refine materials as new information becomes available in Nepal and globally • Interest by donors in conducting operations research to obtain data on effectiveness of WASH strategies	• Not much data exists on the effectiveness of WASH and behavior change interventions, specifically in the Nepali context
Leadership and coordination Mechanism	• Recent interest of donor organizations in the control of cholera in Nepal due to the 2015 earthquakes and their commitment to support the EDCD in their response and control efforts • Increasing attention to cholera control by the government due to 2016 outbreak and response	• No mechanism for an 'emergency' response, which bypasses the typical approval pathways for rapid interventions at the household and community level • No clear cut mechanism for multi-sectoral coordination

3. Preparedness and Response Plan

This Preparedness and Response Plan details the actions necessary to strengthen the existing surveillance of and response to cholera outbreaks. It describes a series of strategies to reduce the risk of transmission of and mortality due to *Vibrio cholerae*.

3.1 Main Objective

Improve the overall health status of the Nepali population by reducing the incidence of cholera and eliminate cholera deaths in Nepal.

3.2 Specific Objectives

1. To prevent the spread of AGE/Cholera
2. To eliminate mortality from AGE/Cholera
3. To ensure coordinated and collaborative AGE/Cholera preparedness and response
4. To ensure a rapid response mechanism during an outbreak of AGE/Cholera

3.3 Strategies for Preparedness and Response

(1) Prevent spread of AGE/Cholera outbreaks

(1.1) Surveillance and early warning

Hospital Reporting

Passive, hospital-based surveillance of suspected cases of epidemic-prone diseases is being instituted as per standard protocol at all district/Zonal/ regional/central hospitals, which serve as sentinel sites under the Early Warning and Response System (EWARS). Each hospital will report the diarrhea cases registered at emergency and OPD departments to their District (Public) Health Offices and the EWARS focal point at EDCD. In case of an epidemic, daily reporting will be done by phone. D(P)HO staff will visit the emergency and OPD departments and collect aggregated information on a standardized form (**Annex 1**). Rumor verification and data monitoring will also take place at district level. Data will be reported from the D(P)HO to the central level (HMIS); reports from each emergency and OPD department will be consolidated into a central database (EWARS). Reporting from the EWARS sentinel sites will be monitored closely by the EDCD surveillance team to ensure that it is accurate and timely.

In an outbreak situation, EWARS should be expanded to additional health facilities beyond designated sentinel sites. The expansion sites should be decided upon before the monsoon season and districts should have a plan for the expansion at least one month prior.

Female Community Health Volunteers working in the districts should be oriented on reporting deaths from AWD in the community that may not have been treated at a health facility to the DPHO. These messages should be continued throughout the monsoon season and be increased in the event of an outbreak in the district or a neighboring district.

Laboratory Surveillance

Rapid Diagnostic Tests (RDT) will be used as point of care diagnosis at the health facility level. Each district will be provided a stockpile of RDTs to distribute to the appropriate facilities as needed. These stockpiles will be prepared prior to the beginning of the cholera season. Methods on how to preform the rapid test are available in **Annex 2**. If culture facilities are not available in the health facility or district level facilities, samples will be collected and stored in Cary Blair Transport Media and transported to the National Public Health Laboratory (NPHL) as soon as possible for confirmation. No less than 10% of cases should be confirmed at

the hospital or national level. In the event that transport media is not available, stool samples can also be stored on filter paper and sent to the National Lab for confirmation by PCR or other molecular studies (methods for sample collection on filter paper can be found in **Annex 2**). All Laboratory results at the health facility or district level facilities should be reported to EWARS as well as the NPHL.

Antibiotic Resistance

In order to provide the best care possible to cholera patients, antibiotic resistance will be monitored. Any facility that performs cholera culture, whether it be hospital lab or NPHL, should test samples positive for cholera for antibiotic resistance. This should be conducted for up to 10 positive samples every two weeks throughout the outbreak. These results should be sent to EDCD as part of regular cholera surveillance reporting. In the event that a new resistance pattern is seen and national antibiotic recommendations need to be updated, the EDCD will handle the dissemination.

Assessments

Annual assessments of laboratory capacity and training of staff will take place at the district level. DPHOs will report to the NPHL whether their district has the capacity for culturing cholera and whether they have culture materials (TCBS and antisera), transport media, filter paper, and RDTs in stock. Reporting on stocks will take place monthly. Resources will be sent to these districts to full-fill their needs as necessary prior to the cholera season. Stocks will be replenished throughout the season as necessary. Coordination meetings with surveillance stakeholders will take place throughout the season.

Reporting

Situation reports will be published weekly by EDCD. This will be increased to daily during an outbreak. An alert will be issued and investigated as potential cholera by EDCD in the event of an adult death with AWD, or a cluster (>3 cases) of AWD with severe dehydration.

Key Activities for Surveillance and Early Warning
Plan for the expansion of EWARS and ensure it is functional in all its current facilities / sentinel sites
Provide a list of sites for expansion of EWARS for each district in case an outbreak occurs
Coordinate meetings with appropriate NGOs and other partners in surveillance efforts
Conduct training for health facility staff (at minimum annually) at the community and district levels regarding surveillance, case definition, data flow, and outbreak response
Establish outbreak response, rumor verification, and monitoring of data at district level
Orient FCHVs on identifying potential cholera cases and deaths in the community
Publish and disseminate weekly (daily during an outbreak) updates via AGE/ Cholera Situation Reports
Conduct laboratory capacity assessments and distribute RDTs to all DPHOs for confirmation of cases at all levels of the health care delivery system

(1.2) Water, Sanitation and Hygiene (WASH)

Community Level WASH Interventions

Preventive water, sanitation and hygiene (WASH) interventions will be specifically targeted to the household, community

including food vendors, school, work-place and health facility levels. The following list of interventions will be stockpiled at the national level for deployment to districts in need throughout the cholera season: Piyush, aquatabs, soap, hygiene kits, and educational posters and pamphlets.

Specific WASH activities will take place prior to the cholera season in high-risk areas in each district. These include standardized radio messaging on health behaviors and in-person messaging campaigns in any internal displacement camps. The DPHO will also be responsible for ensuring that water treatment, such as aquatabs, are available locally prior to the season, and that solid waste is handled appropriately. DPHO will also encourage construction of household toilets and hand washing facilities and/or adequate maintenance.

Water Supply Interventions

Specific interventions concerning access to drinking water will be established by performing routine analysis of the existing water supply infrastructure, storage, and distribution network. Water supply stakeholders will be invited as key members of the Steering Committee for Enteric Diseases to provide reports on the system prior to the monsoon season. The Department of Water Supply and Sanitation (DWSS) and its district offices will be engaged to provide routine water quality monitoring in the form of free residual chlorine and fecal coliform tests. Any drinking water sources found to be unfit for drinking will be followed up on by the MOH. Resources will be provided to the DWSS laboratory for culturing of *Vibrio cholera.* If any source is found to contain *Vibrio cholerae*, the DPHO will deploy individuals to ensure the source is not used by the public until the issue is solved. Any source found positive for *V. cholerae* by the DWSS, will be confirmed by NPHL.

Key Activities for WASH Response
Chlorinate all piped drinking water systems and water tankers
Regularly monitor systems prior to the season and more frequently during outbreaks, including provision of chlorination materials and training where required
Train on point of use drinking water treatment at the household level in high risk areas
Implement a food safety campaign targeting both food vendors and households through social mobilization and mass media channels
Practice hand-washing with soap through health messaging campaigns at critical times
Ensure proper collection and management of solid waste
Support community led sanitation activities to encourage households and health posts to rebuild/build toilets and hand-washing facilities
Maintain adequate sanitation and hand-washing facilities in households
Pre-position WASH materials at the local level to enable rapid scale-up of interventions in new areas, and continuity in existing areas, in the event of an outbreak
Stockpile adequate WASH equipment and ensure materials are available in Kathmandu to be deployed when necessary

(1.3) Immunization with oral cholera vaccine (OCV)

OCV Strategy

Oral cholera vaccines (OCV) are safe, effective, and acceptable. They present a tool for cholera control that supplements, but vaccine does not replace, existing cholera control measures such as WASH

interventions. Three WHO pre-qualified oral cholera vaccines are available through the Global Stockpile; two are available for direct purchase.

Vaccination of populations can either be pre-emptive, before the cholera season in evidence-based, pre-determined hot-spot areas, or as a reactive action during an outbreak in order to limit and reduce spread of the disease. Reactive vaccination may be carried out in an area at risk for cholera and adjacent to an area with ongoing transmission. Due to the limited amount of vaccine available in the country at this time and limited availability of data on which to base targeting of pre-emptive vaccination, a reactive approach is favored for the country at this time.

Vaccination will be used for:

- Prevention of potential cholera outbreaks where essential services to prevent the spread of *Vibrio cholerae* in the environment (adequate clean water, sanitation and hygiene) and health care are disrupted or destroyed, mobile populations residing in crowded settings, or areas at high-risk for cholera outbreaks. Any pre-emptive OCV campaign should be conducted at least two months prior to the monsoon season with two doses administered two weeks apart.

- Reducing the spread of cholera and limiting mortality in communities neighboring a current outbreak (communities across borders or linked by river systems or water and sanitation systems). A reactive vaccination strategy (vaccination within a 100-meter radius around an index cases) can be applied in outbreak situations with a single dose of OCV.

If possible, a stockpile of vaccine will be created at the national level (ideally, 50,000 doses). This stockpile would be in place prior to the monsoon season and provide doses for reactive vaccination. This stockpile would be replenished as the vaccine was used, and could also provide a buffer in the event more vaccine needs to be obtained for larger outbreaks,

Planning

Detailed micro-planning will take place at the periphery level. Training will be conducted for FCHVs and health workers prior to the start of the season, and refresher trainings will take place as needed. Standard forms will be available in each district. Media will be oriented to the use of the OCV to ensure that a positive message is sent to the public. Radio messaging will also take place at the district level to ensure the population has access to accurate information on when/ where/why campaigns are taking place.

Reporting

Vaccination teams must also use tally sheets (**Annex 3**) to record the number of people vaccinated per day, at each vaccination site by each team. FCHVs will monitor the population for any adverse events post-vaccination and report results to the district level using a standard form (**Annex 4**). Number of new cases that appear in the vaccinated area will also be reported for analysis at the national level.

Incorporation of Health Education

OCV should not be used as a stand -alone intervention. Integrated information, education and communication materials will be distributed along with vaccine and will be available in each district prior to the season (**Annex 5**).

Key Activities for Immunization
Select areas for pre-emptive vaccination if adequate information is available to support a hot-spot or hot-pop
Ensure a stockpile of vaccine is in place prior to the beginning of monsoon season
Distribute integrated information, education, and communication (IEC) materials for OCV and safe hygiene practices, (i.e. hand washing with soap)
Develop a detailed micro-plan for vaccine delivery and ensure it is available and understood at the periphery level
Conduct training for FCHVs and health workers on OCV and hygiene promotion
Ensure availability of guidelines, forms, and IEC materials for OCV campaigns at the district level
Conduct media orientation (at both central and district levels) to mitigate rumor/ negative coverage of OCV campaigns
Air radio/FM messages on OCV campaigns using district level radio stations
Ensure proper documentation and monitoring and evaluation of cholera campaigns by providing standard materials to each DPHO

(1.4) Behavior Change Communication (BCC) and Social Mobilization

Extensive community mobilization and behavior change communication activities are required to prevent and mitigate AGE/ cholera by encouraging safe hygiene practices. Existing networks of partner NGOs and FCHVs in affected districts work with district (public) health offices to come up with a detailed activity plan and will then be mobilized to disseminate key messages and orient households at community level prior to the cholera season. During the cholera season, FCHVs, as the hub of community mobilization, will be given simple communication guideline and then activated to deliver regular interpersonal communication sessions on key health/WASH behaviors, as well as distribute essential health/WASH items such as ORS, zinc, and soap to hot-spots and hot-pops in their district. WASH promotion will also take place during the season at schools and community gatherings.

Key Activities for BCC and Social Mobilization
Develop a detailed activity plan and align with other stakeholders working in health and WASH sectors at the district level
Involve NGO partners working in health and WASH and support them in their promotion of safe hygiene practices
Orient teachers and school children on WASH
Promote WASH through door to door mobilization of FCHVs in the community
Distribute simple communication guidelines on key hygiene behaviors, case detection, and referral to community-level social mobilizers (identified by DPHOs)
Promote key health behaviors during community gatherings

(1.5) Community Level Interventions

Community-based interventions for health, nutrition and WASH can be integrated and promoted into all community-based activities in a variety of sectors through community-based actors to target greater outreach to the communities. The following interventions will be planned:

- Health and hygiene promotion (distribution of brochures)
- Water testing

- Distribution of soap and Piyush at high risk areas
- Miking in high-risk areas
- Active case finding and early detection by FCHVs, including referral to health facilities
- Communication on use of household water treatment and ORS at home

Key Activities at the Community Level
Identify all actors to deliver integrated services: community health workers, village health workers, hygiene promoters, social mobilisers, traditional birth attendants, Red Cross volunteers etc.
Conduct training for community level actors in health education and hygiene promotion, active case finding, early case detection, use of household water treatment and ORS at home, and referral to health facilities
Distribute IEC materials for safe hygiene practices, household water treatment, and messages on when to start home treatment with ORS and/or to seek immediate treatment to the community

(2) Reduce the mortality from AGE/cholera

(2.1) Standardized Case Management

Assessment

Capacity building for identification, case management, reporting, referral, and infection control in health care settings will take place in all levels of health facilities annually. EDCD will coordinate capacity building activities to avoid duplication and ensure that critical gaps are filled prior to the cholera season. The EDCD will work to provide a digital resource center where stakeholders can access forms, guidelines, dehydration algorithms and other materials.

Treatment of Cholera Cases

Cholera is an easily treatable disease. Treatment centers around rehydration. The prompt administration of oral rehydration salts to replace lost fluids (5ml/kg/hour) nearly always results in the patient being cured. All district level health facilities will be activated and medical officers will be trained for treatment prior to the start of the cholera season. In cases of severe dehydration or hypovolemic shock due to diarrhea (**Table 2**), intravenous administration of fluids (Ringers Lactate; **Table 3**) may be required to save the patient's life. These patients should be monitored every 30 minutes. After the first 30ml/kg of fluids have been given the patient's radial pulse should be strong and the patient's blood pressure should return to normal. If the pulse is not strong, IV fluid administration should be continued. Adults should be reassessed after 3 hours and infants after 6 hours of receiving fluids and start ORS (about 5 ml/kg/hr) as soon as patient can drink safely.

Table 2: Assessment of Dehydration

	Plan A	Plan B	Plan C
Observe: Condition Eyes Tears Mouth/ Tongue Thirst	Well Alert Normal Present Moist Normal	Restless, Irritable Sunken Absent Dry Thirsty, drinks eagerly	Lethargic, coma Very sunken Absent Very dry Unable to drink
Feel: Skin Pinch	Goes back quickly	*Goes back slowly	*Goes back very slowly
Decide:	Patient has no signs of dehydration	At least 2 signs, including one '*' sign: Some dehydration	At least 2 signs, including one '*' sign: Severe dehydration

Table 3: Estimation of Ringers Lactate IV for patients with Severe Dehydration		
Age	**First, Administer 30ml/kg in**	**Then give 70ml/kg in**
Infants (<12 months)	1 hour	5 hours
1 year and over	30 minutes	2.5 hours

Antibiotics should be given to any patient with cholera who comes for treatment and the preferred antibiotic is doxycycline (currently circulating strain must be checked for resistance). In the case of resistance to doxycycline, azithromycin should be used. Azithromycin should also be used for pregnant women and children under eight years. By decreasing the duration of diarrhea and stool volume, antibiotic use will result in more rapid recovery and shorter lengths of inpatient stay, both of which contribute to optimizing resource utilization in an outbreak setting. Hydration kits will be deployed to District Public Health Offices for their EWARS sentinel sites well before the typical cholera season, and will be replenished immediately upon use.

For more detailed information on cholera treatment guidelines please refer to the WHO's "The Treatment of Diarrhoea: A manual for physicians and other senior health workers." This document was last revised in 2005 and is available online.

Discharge of Cholera Patients

Suspected cholera patients should remain at the health facility until diarrhea and vomiting have stopped (expected within 24 hours). Even after dehydration is corrected, additional fluids may be needed to compensate for ongoing fluid losses. The patient should be told to return to the health facility if they experience an increased number of stools, loss of appetite, excessive thirst, repeated vomiting, fever, or blood in stool.

Key Activities for Case Management
Annual Training of Trainers (TOT) for AGE/ Cholera management and subsequent training of health workers
Monitor training activities at the district level
Create a Digital Resource Center accessible to all districts and partners through the EDCD
Support supervision, monitoring visits, and quality assurance through standardized practices
Distribute case management guidelines and algorithms for assessing dehydration and managing patients, including the identification and management of dehydration for malnourished children

(2.2) Supplies and Logistics

Stockpile

Medical supplies including Interagency Diarrheal Disease Kits (IDDKs), additional ORS, antibiotics, and other supplies will be strategically pre-positioned by EDCD, WHO and UNICEF at the regional level. There will be an additional stockpile of IDDKs in Kathmandu.

Distribution

Regular tracking and monitoring will take place and each region will receive supplies based on the number of EWARS sentinel sites and expansion sites in that region. Supplies will be distributed no later than one month prior to the monsoon season. Additional supplies will be sent from the national stockpile in Kathmandu as necessary during outbreak situations.

Key Activities for Supplies and Logistics
Map necessary supplies annually to address current needs, including a distribution plan
Procure additional supplies as needed for agreed minimum level preparedness
Ensure timely distribution of available supplies based on need and risk in affected areas
Regularly track and monitor inventory and replenish supplies as needed

(3) Coordination and Collaboration Surrounding Cholera Preparedness and Response

Steering Committee for Enteric Diseases and Task Force for Cholera Control

The Epidemiology and Disease Control Division of the Department of Health Services (EDCD) will take the lead and provide overall coordination and collaboration of AGE/cholera prevention, preparedness, and response activities. They will disseminate timely surveillance information through an AGE/cholera Situation Report to enable rapid response by all stakeholders. The existing high-level Steering Committee for Control of Enteric Diseases will guide the decision making for the control of enteric diseases in Nepal and meet one moth prior to each monsoon season to discuss plans for cholera preparedness and response. This committee will also ensure that all districts have the necessary resources and are aware of reporting mandates. The Task Force for Cholera Control, a sub-committee of the abovementioned Steering Committee is chaired by Director of EDCD and includes representation from the WASH cluster, WHO, UNICEF, GTA, and relevant INGOs/NGOs in addition to the Ministry of Health (MoH). They will be responsible for mapping available resources to identify gaps prior to the season. It will be responsible to ensuring any decisions from the Steering Committee are implemented accordingly. The task force will meet at least monthly during the monsoon season to monitor the response and to ensure a solid and well-coordinated response mechanism for immediate action. The task force will monitor progress of outbreaks and review the prevention, preparedness and response plan on a yearly basis to adjust to prevailing situation, and will tailor the response more frequently during an outbreak if absolutely necessary.

Situation Reports

As a means of rapidly disseminating information on the current cholera situation to all interested parties, the EDCD will publish a weekly situation report during the monsoon season detailing AGE and cholera cases. This report will include information on both suspected and confirmed cases, and include the number of cases reported by each EWARS sentinel site. A map detailing the geographic distribution of cases will also be included in the report to assist in developing response activities. An example of the Situation Report can be found in **Annex 6**. In the event of an outbreak situation, this report will be updated and distributed on a daily basis.

Key Activities for Coordination and Collaboration
Map available resources and existing gaps (funds, supplies, partner contact details, response plans etc.) prior to the season to ensure preparedness
Conduct regular meetings of the Task force for Cholera Control to obtain updates on the AGE/cholera situation, surveillance and control operations, status of essential supplies, and gaps in resources

Key Activities for Coordination and Collaboration, continued
Share information through a weekly AGE/ Cholera Situation Report sent by email from EDCD with a summary of the current AGE/Cholera numbers (daily in the case of an outbreak)
Discuss possible field level (district and below) coordination and collaboration of response through the government health system and other stakeholders to ensure a response can take place as soon as possible

(4) Rapid Response Mechanism

Cholera has the ability to spread quickly, particularly when a contaminated drinking water source is used by a large population. Therefore, it is essential that the government response takes place as soon as possible, and preferably, within 24 hours of a case being reported. Investigations should be conducted whenever a single case meets the clinical definition since the illness is likely to be more widespread than a single case (given the majority of cholera cases are mild or asymptomatic). Previous experience has shown that implementing interventions such as a WASH or vaccination campaign in response to a case of cholera under the current system can take weeks. Therefore, a mechanism will be put in place within the existing government system to ensure the response activities outlined in the national plan can be initiated within this critical 48-hour window. A government official will be selected who has the authority and responsibility to give the "green light" for implementation of community level campaigns to control cholera. Districts should have agreed upon action plans for such campaigns at least two months prior to the start of the season to avoid delays. Stockpiles of necessary supplies at the district, regional, and national levels will be utilized to carry out the response. Plans will be made at the district level for when/where supplies will be retrieved and who will be responsible for delivering them.

Rapid Response Teams

Rapid Response Teams (RRT) should be formed at the central, regional, and district levels. These teams will be trained annually and be on stand-by during the monsoon season to conduct field investigations, deliver interventions, and ensure stool specimens are send to the lab for confirmation. At the central level, the Director of EDCD will serve as the chair, the Chief of the Epidemiology Section will be the focal point, and membership will also contain representatives from the National Public Health Laboratory, Child Health Division, Logistic Management Division and other sections of EDCD. The Regional Director will be the focal point for regional level RRT and the other members will include a medical officer, a malaria/public health officer, a health assistant, a health education officer, a statistics officer, the immunization supervisor, a lab technician and a public health nurse. The district level RRT will be chaired by the Chief of the DPHO, with a health assistant from the DPHO as the focal point. Other members of the district level RRT will include the immunization supervisor, a health education technician, a medical officer, a public health nurse, a lab technician, a statistician, a medical recorder and a family planning assistant.[29]

When RRTs are mobilized, the field team should consist of at least one public health/clinical officer, one lab technician, and one health educator. FCHVs from the municipalities/VDCs can be included as necessary, dependent upon the size of the outbreak. The duties of the rapid response team are to:

- Verify reported cases
- Investigate new cases
- Obtain lab specimens for confirmation
- Identify hot-populations (high-risk groups)
- Investigate water source contamination
- Assess local capacity to respond (case management and community control measures)
- Implement control measures (including WASH and OCV)
- Provide emergency treatment and supplies
- Collect line listing of information on cases for analysis
- Report findings to EDCD

Line Listing and Risk Factor Collection

A line listing should be made available to rapid response teams by EWARS sentinel sites in order to identify cases for household investigation. It is essential that this line listing include identifying information for the patient (name, age, address, telephone number). For each case, the rapid response team should collect and analyze data on the following risk factors:

- Recent travel history
- Contact with persons with diarrhea
- Recent attendance at a crowded event
- Water sources for drinking, bathing, and cleaning kitchen utensils
- Food history (consuming raw fruits, vegetables or juices, eating room-temperature food)
- Occupation

An example of a risk factor data collection form can be found in **Annex 7**.

Key Activities for a Rapid Response
Designate a government official responsible for emergency implementation of response activities
Prepare district level plans for the conduct of community level WASH and reactive vaccination campaigns at least two months prior to the monsoon season
Map where supplies will be retrieved and who will deliver them for rapid implementation of the cholera response
Train RRTs annually on response activities
Collect and analyze data from the line listings and household investigations of all cholera cases

4. Plan of Action

SN	Key Activities	Period			Responsibilities
		Pre-monsoon	During Monsoon	Post-Monsoon	
1	**Surveillance and Early Warning Activities**				
i	Plan for the expansion of EWARS and ensure it is functional in all its current facilities / sentinel sites				EDCD/DPHO/Health Facilities
ii	Provide a list of sites for expansion of EWARS for each district in case an outbreak occurs				EDCD/DPHO/Health Facilities
iii	Coordinate meetings with appropriate NGOs and other partners in surveillance efforts				EDCD/DPHO/I/NGO partners
iv	Conduct training for health facility staff (at minimum annually) at the community and district levels regarding surveillance, case definition, data flow, and outbreak response				EDCD/DPHO/I/NGO partners
v	Orient FCHVs on identifying potential cholera cases and deaths in the community				DPHO/ Health Facilities
vi	Establish outbreak response, rumor verification, and monitoring of data at district level				EDCD/DPHO
vii	Publish and disseminate weekly (daily during an outbreak) updates via AGE/Cholera Situation Reports				EDCD
viii	Conduct laboratory capacity assessments and distribute RDTs to all DPHOs for confirmation of cases at all levels of the health care delivery system				EDCD/NPHL/DPHO/I/ NGO partners
2	**WASH Activities**				
i	Chlorinate all piped drinking water systems and water tankers				DWSS/Water vendors/I/ NGO partners
ii	Regularly monitor systems prior to the season and more frequently during outbreaks, including provision of chlorination materials and training where required				DWSS/EDCD/District Line Agencies

SN	Key Activities	Period			Responsibilities
		Pre-monsoon	During Monsoon	Post-Monsoon	
iii	Train on point of use drinking water treatment at the household level in high risk areas				DPHO/WSSDO/ Community People
iv	Implement a food safety campaign targeting both food vendors and households through social mobilization and mass media channels				EDCD/DFTQC/DPHO/ Municipalities/I/NGO partners
v	Practice hand-washing with soap through health messaging campaigns at critical times				EDCD/NHEICC/DPHO/ Municipality/Media/I/ NGO Partners
vi	Ensure proper collection and management of solid waste				Municipality/Private sectors/I/NGOs
vii	Support community led sanitation activities to encourage households and health posts to rebuild/build toilets and hand-washing facilities				Municipality/VDC/I/ NGOs/ Community People
viii	Maintain adequate sanitation and hand-washing facilities in households				Municipality/VDC/I/ NGOs/ Community People
ix	Pre-position WASH materials at the local level to enable rapid scale-up of interventions in new areas, and continuity in existing areas, in the event of an outbreak				EDCD/DPHO/WSSDO/I/ NGO partners
x	Stockpile adequate WASH equipment and materials are available in Kathmandu to be deployed when necessary				EDCD/LMD/I/NGO partners
3	**Immunization Activities**				
i	Select areas for pre-emptive vaccination if adequate information is available to support a hot-spot or hot-pop				EDCD/CHD/DPHO/local health facilities/I/NGO partners
ii	Ensure a stockpile of vaccine is in place prior to the beginning of monsoon season				EDCD/CHD/LMD

SN	Key Activities	Period			Responsibilities
		Pre-monsoon	During Monsoon	Post-Monsoon	
iii	Distribute integrated information, education, and communication (IEC) materials for OCV and safe hygiene practices, (i.e. hand washing with soap)				EDCD/CHD/DPHO/local health facilities/I/NGO partners
iv	Develop a detailed micro-plan for vaccine delivery and ensure it is available and understood at the periphery level				EDCD/CHD/DPHO/local health facilities/I/NGO partners
v	Conduct training for FCHVs and health workers on OCV and hygiene promotion				EDCD/CHD/DPHO/local health facilities/I/NGO partners
vi	Ensure availability of guidelines, forms, and IEC materials for OCV campaigns at the district level				EDCD/CHD/DPHO/I/ NGO partners
vii	Conduct media orientation (at both central and district levels) to mitigate rumor/negative coverage of OCV campaigns				EDCD/DPHO/I/NGO partners
viii	Air radio/FM messages on OCV campaigns using district level radio stations				EDCD/DPHO/I/NGO partners/local medias
ix	Ensure proper documentation and monitoring and evaluation (M&E) of cholera campaigns by providing standard materials to each DPHO				EDCD/DPHO/I/NGO partners
4	**Social Mobilization Activities**				
i	Develop a detailed activity plan and align with other stakeholders working in health and WASH sectors at the district level				DWSS/EDCD/DPHO/ WSSDO/I/NGO partners
ii	Involve NGO partners working in health and WASH and support them in their promotion of safe hygiene practices				EDCD/DPHO/WSSDO/I/ NGO partners
iii	Orient teachers and school children on WASH				EDCD/DPHO/WSSDO/ VDC/NGO partners
iv	Promote WASH through door to door mobilization of FCHVs in the community				EDCD/DPHO/WSSDO/ Municipality/VDC/I/NGO partners

SN	Key Activities	Period			Responsibilities
		Pre-monsoon	During Monsoon	Post-Monsoon	
v	Distribute simple communication guidelines on key hygiene behaviors, case detection, and referral to community-level social mobilizers (identified by DPHOs)				EDCD/DPHO/WSSDO/ Municipality/VDC/I/NGO partners
vi	Promote key health behaviors during community gatherings				EDCD/DPHO/WSSDO/ Municipality/VDC/I/NGO partners
5	**Community Level Activities**				
i	Identify all actors to deliver integrated services: community health workers, village health workers, hygiene promoters, social mobilisers, traditional birth attendants, Red Cross volunteers etc.				EDCD/DPHO/ Municipality/VDC/I/NGO partners
ii	Conduct training for community level actors in health education and hygiene promotion, active case finding, early case detection, use of household water treatment and ORS at home, and referral to health facilities				EDCD/DPHO/ Municipality/VDC/Local Health Facilities/I/NGO partners
iii	Distribute IEC materials for safe hygiene practices, household water treatment, and messages on when to start home treatment with ORS and/ or to seek immediate treatment to the community				EDCD/DPHO/ Municipality/VDC/Local Health Facilities/I/NGO partners/FCHVs
6	**Case Management Activities**				
i	Annual Training of Trainers (TOT) for AGE/Cholera management and subsequent training of health workers				EDCD/DPHO/I/NGO partners
ii	Monitor training activities at the district level				EDCD/DPHO

SN	Key Activities	Period			Responsibilities
		Pre-monsoon	During Monsoon	Post-Monsoon	
iii	Create a Digital Resource Center accessible to all districts and partners through the EDCD				EDCD/DPHO/I/NGO partners
iv	Support supervision, monitoring visits, and quality assurance through standardized practices				EDCD/DPHO/I/NGO partners
v	Distribute case management guidelines and algorithms for assessing dehydration and managing patients, including the identification and management of dehydration for malnourished children				EDCD/DPHO/Local Health Facilities/I/NGO partners
7	**Supplies and Logistics Activities**				
i	Map necessary supplies annually to address current needs, including a distribution plan				EDCD/LMD/DPHO/ Local Health Facilities / NGO partners
ii	Procure additional supplies as needed for agreed minimum level preparedness				EDCD/LMD/DPHO/I/ NGO partners
iii	Ensure timely distribution of available supplies based on need and risk in affected areas				EDCD/LMD/DPHO/I/ NGO partners
iv	Regularly track and monitor inventory and replenish supplies as needed				EDCD/LMD/DPHO
8	**Coordination and Collaboration Activities**				
i	Map available resources and existing gaps (funds, supplies, partner contact details, response plans etc.) prior to the season to ensure preparedness				EDCD/LMD/CHD/DWSS/ WHO/UNICEF/DPHO/I/ NGO partners
ii	Conduct regular meetings of the Task force for Cholera Control to obtain updates on the AGE/ cholera situation, surveillance and control operations, status of essential supplies, and gaps in resources				EDCD/LMD/CHD/DWSS/ WHO/UNICEF/DPHO/I/ NGO partners

SN	Key Activities	Period			Responsibilities
		Pre-monsoon	During Monsoon	Post-Monsoon	
Iii	Share information through a weekly AGE/Cholera Situation Report sent by email from EDCD with a summary of the current AGE/Cholera numbers (daily in the case of an outbreak)				EDCD/LMD/CHD/DWSS/ WHO/UNICEF/DPHO/I/ NGO partners
Iv	Discuss possible field level (district and below) coordination and collaboration of response through the government health system and other stakeholders to ensure a response can take place as soon as possible				DPHO/I/NGO partners
9	**Rapid Response Activities**				
i	Designate a government official responsible for emergency implementation of response activities				EDCD/DPHO/Health facilities
ii	Prepare district level plans for the conduct of community level WASH and reactive vaccination campaigns at least two months prior to the monsoon season				EDCD/LMD/CHD/DWSS/ WHO/UNICEF/DPHO/I/ NGO partners
iii	Map where supplies will be retrieved and who will deliver them for rapid implementation of the cholera response				EDCD/CHD/DWSS/WHO/ UNICEF/DPHO/I/NGO partners
iv	Train RRTs annually on response activities				EDCD/DPHO/I/NGO partners
v	Collect and analyze data from the line listings and household investigations of all cholera cases				EDCD/DPHO

5. Monitoring and Evaluation of Surveillance and Response

Conducting routine monitoring and evaluation is important in ensuring an effective and efficient surveillance and response system year after year (WHO). Monitoring and evaluation of the successful implementation of this plan will be led by EDCD at the EWARS sentinel sites and by the DPHOs in coordination with EDCD at periphery level health facilities.

According to the WHO Guide to Monitoring and Evaluation of Communicable Disease Surveillance and Response Systems, monitoring in this context refers to "the routine and continuous tracking of the implementation of planned surveillance activities and of the overall performance of surveillance and response systems," and evaluation refers to the "periodic assessment of the relevance, effectiveness and impact of activities" based on the objectives set forth for those activities. [30] These activities must be implemented in a manner that allows for adjustment of the plan on an annual basis. The Steering Committee for Enteric Diseases, chaired by the Director of the EDCD, will review the results of this evaluation during their post-monsoon session and recommend any necessary revisions to the plan.

Indicators

A series of indicators have been outlined in **Annex 8** and are specified for surveillance, response, and laboratory activities. The EDCD is responsible for collecting data on these indicators and analyzing results for dissemination to the Steering Committee. Explanations of results as well as specific recommendations should be provided.

Timing the evaluations

Evaluations will take place annually during the post-monsoon season after cases have subsided. However, the Task Force for Cholera Control should also perform interim evaluations may be performed to track progress determine whether the program is on target with the goals outlined in this plan and to implement changes if needed. This will be particularly important for monitoring stockpiles of supplies throughout the season. It will also ensure that stakeholders can be held responsible for their activities as outlined in the plan of action.

Key Activities for Monitoring and Evaluation
Track progress of implementation of planned activities
Identify problems in the system in order to institute corrective measures in a timely manner
Track stocks of key resources to avoid delays in response
Ensure that all parties are held responsible and accountable for their defined activities as outlined in the Plan of Action
Collect and analyze data on the outlined indicators
Disseminate results to the Steering Committee
Provide explanations for achievements and failures in the system
Provide specific recommendations for improving the system

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Annexes

ANNEX 1a: Hospital Reporting Form

Hospital	Patient ID	OPD/Eme/ IPD	Name	Age (Year)	Sex	Phone	Name Of Guardian	Date Of Admission	District	VDC	Ward	Village Or Tole

Provisional diagnosis AGE or Cholera	Stool sample collected (Y/N)	Outcome	RDT Performed	Culture Performed	Result RDT	Result Culture	Date of Lab Result	Sample sent to NPHL (Y/N)	Date Sent to NPHL

ANNEX 1b: Variable Definitions

Variable Name	Description	Codes
Hospital	Name of the hospital	Text
Patient ID	Registration number of the patients or if not available registration number as specified in the ER registers	Num
Name of Patient	Name of the patient	Text
Age	age of the patient	Num
Sex	sex of the patient	M/F
Phone	Phone number (mobile) of the patient	Num
Name of Guardian	Name of the father or mother or relatives	Text
Date of Admission	Date of admission in ER or OPD or date of registration in wards	Date
OPD/Eme/IPD	Please select type of patient registration (OPD/IPD/Emergency).	OPD, EME, IPD
District	District of current residence	Coded values
VDC	VDC of current residence	VDC codes
Ward	Number of the ward of the current residence	Num
VillageOrTole	Name of the Village or Tole	Text
ProvisionalDiagnosis	Provisional diagnosis during admission	AWD (Acute Watery Diarrhoea) ; SC(Suspected cholera)
StoolCollected	Was a stool sample collected in admission?	Y/N
Outcome	Outcome of the disease at discharge	1. Treatment; 2.cured;2.referred;3. death;4.Unk
RDT	Was an RDT for cholera performed?	Y/N
Culture	Was culture performed	Y/N
ResultRDT	Result of cholera RDT	Positive/Negative
ResultCulture	Result of Culture for cholera at hospital	Positive/Negative
DateLabResult	Date it was tested in the laboratory of the hospital	Date
DateResultNPHL	Date it was tested in NPHL	Date
Sample sent to NPHL (Y/N)	Was the sample sent to NPHL?	Y/N
DateSentNPHL	The date the sample was sent to NPHL	date

ANNEX 2: Methods for Using the Rapid Diagnostic Test with Enrichment Step

Fecal Specimen Collection Procedure

1 For collection of fecal specimens, the laboratory technician must collect a recently discharged or fresh stool sample from the patient. Prepare necessary supplies including labeled stool container, gloves, plastic spoon, and 2 plastic bags at the time of presentation. Use the plastic spoon to collect 3-4 spoons of stool and place it in the labeled stool container properly. If the stool is watery, use container directly to collect approximately 5 ml of stool.

2 If a diarrheal stool sample is not available, the health facility clinical staff should collect a rectal swab. To collect a rectal swab, take verbal consent, insert swab 1-1.5 inches into the rectum and gently rotate. The swab should be visibly stained with stool. Then insert the swab deep into a tube of APW, break off the tip, and close the cap.

Enriched Cholera RDT

1 Ensure appropriate PPE is worn by health facility staff processing the specimens.

2 Immediately upon receipt of the fecal specimen, dip a cotton-tipped wooden stick in the fecal specimen and place in APW media for **6 hours** (minimum of **5** to a maximum of **18** hours).

APW must be inoculated on the day of fecal specimen collection OR Cary-Blaire must be inoculated from the original specimen.

3 Using a pipette, collect a small amount of specimen-enriched APW from the top of the APW tube. Do not shake the APW tube prior to collection. Add 2-4 drops of the specimen-enriched APW into kit's test tube.

4 Place a Crystal VC dipstick into the test tube and wait for exactly 15 minutes (do not exceed 15 minutes).

5 Read the dipstick at 15 minutes, verifying that the positive control band is visible to ensure quality of the dipstick.

If the specimen is dipstick POSITIVE

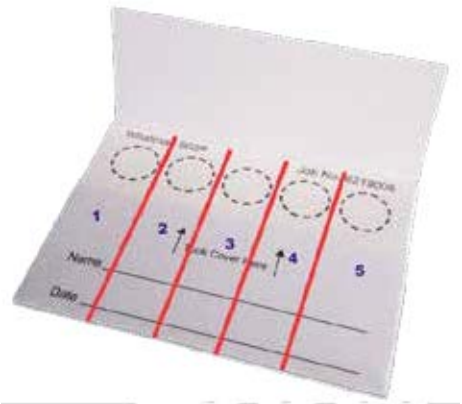
1 Inoculate the first 10 positive specimens at each individual facility into Cary-Blair transport media to be sent for culture. To do so:

1 Dip cotton-tipped wooden stick into the fresh stool/specimen-enriched APW and then **stab** stick into Cary-Blair transport tube. Cotton tip should be inserted to the bottom of the Cary Blair media.

2 Break off the tip of the cotton-tipped wooden stick and close the tube tightly.

If transport media is not available

1 Blot stool directly onto a Filter Paper card (shown at the right) , filling one circle and labeling with patient id, hospital name, and date. (for further molecular studies)



ANNEX 3: Vaccination Talley Sheet

Cholera Vaccination Tally Sheet by Sex

Sheet N°:.....

Team:..... District:.....

Date:..... Health Zone:.....

Health Area:..... Neighborhood:.....

Number of vaccine vials used:

Utilization rate= Number of people vaccinated x100 / Number of doses used Utilization rate %

	Male				Female			
≥ 1yr - 4 yr	○○○○○ ○○○○○	○○○○○ ○○○○○	○○○○○ ○○○○○	○○○○○ ○○○○○	○○○○○ ○○○○○	○○○○○ ○○○○○	○○○○○ ○○○○○	○○○○○ ○○○○○
	○○○○○ ○○○○○	○○○○○ ○○○○○	○○○○○ ○○○○○	○○○○○ ○○○○○	○○○○○ ○○○○○	○○○○○ ○○○○○	○○○○○ ○○○○○	○○○○○ ○○○○○
	○○○○○ ○○○○○	○○○○○ ○○○○○	○○○○○ ○○○○○	○○○○○ ○○○○○	○○○○○ ○○○○○	○○○○○ ○○○○○	○○○○○ ○○○○○	○○○○○ ○○○○○
	○○○○○ ○○○○○	○○○○○ ○○○○○	○○○○○ ○○○○○	○○○○○ ○○○○○	○○○○○ ○○○○○	○○○○○ ○○○○○	○○○○○ ○○○○○	○○○○○ ○○○○○
	○○○○○ ○○○○○	○○○○○ ○○○○○	○○○○○ ○○○○○	○○○○○ ○○○○○	○○○○○ ○○○○○	○○○○○ ○○○○○	○○○○○ ○○○○○	○○○○○ ○○○○○
Total 1 - 4 yr								
≥ 5 - 14 yr	○○○○○ ○○○○○	○○○○○ ○○○○○	○○○○○ ○○○○○	○○○○○ ○○○○○	○○○○○ ○○○○○	○○○○○ ○○○○○	○○○○○ ○○○○○	○○○○○ ○○○○○
	○○○○○ ○○○○○	○○○○○ ○○○○○	○○○○○ ○○○○○	○○○○○ ○○○○○	○○○○○ ○○○○○	○○○○○ ○○○○○	○○○○○ ○○○○○	○○○○○ ○○○○○
	○○○○○ ○○○○○	○○○○○ ○○○○○	○○○○○ ○○○○○	○○○○○ ○○○○○	○○○○○ ○○○○○	○○○○○ ○○○○○	○○○○○ ○○○○○	○○○○○ ○○○○○
	○○○○○ ○○○○○	○○○○○ ○○○○○	○○○○○ ○○○○○	○○○○○ ○○○○○	○○○○○ ○○○○○	○○○○○ ○○○○○	○○○○○ ○○○○○	○○○○○ ○○○○○
	○○○○○ ○○○○○	○○○○○ ○○○○○	○○○○○ ○○○○○	○○○○○ ○○○○○	○○○○○ ○○○○○	○○○○○ ○○○○○	○○○○○ ○○○○○	○○○○○ ○○○○○
Total 5 - 14 yr								
≥ 15 yr	○○○○○ ○○○○○	○○○○○ ○○○○○	○○○○○ ○○○○○	○○○○○ ○○○○○	○○○○○ ○○○○○	○○○○○ ○○○○○	○○○○○ ○○○○○	○○○○○ ○○○○○
	○○○○○ ○○○○○	○○○○○ ○○○○○	○○○○○ ○○○○○	○○○○○ ○○○○○	○○○○○ ○○○○○	○○○○○ ○○○○○	○○○○○ ○○○○○	○○○○○ ○○○○○
	○○○○○ ○○○○○	○○○○○ ○○○○○	○○○○○ ○○○○○	○○○○○ ○○○○○	○○○○○ ○○○○○	○○○○○ ○○○○○	○○○○○ ○○○○○	○○○○○ ○○○○○
	○○○○○ ○○○○○	○○○○○ ○○○○○	○○○○○ ○○○○○	○○○○○ ○○○○○	○○○○○ ○○○○○	○○○○○ ○○○○○	○○○○○ ○○○○○	○○○○○ ○○○○○
	○○○○○ ○○○○○	○○○○○ ○○○○○	○○○○○ ○○○○○	○○○○○ ○○○○○	○○○○○ ○○○○○	○○○○○ ○○○○○	○○○○○ ○○○○○	○○○○○ ○○○○○
Total ≥15 ry								
Grand total								
	Check a circle for a given dose (1 person vaccinated)				1 square = 100 doses administered			

Utilization	Number of doses received	Extra number of doses received	Total received	Number of remaining doses	Number of doses used
Vaccine doses					

Visial vial monitor

Reading				
Interpretation	Can be used		Can not be used	
Number of VVMs with changed color				

ANNEX 4: OCV Adverse Event Following Immunization (AEFI) Reporting Form

Date of report:		Age:	M /F:
> 3 years old: yes/no	Pregnant: yes/no	Immune compromised: yes/no	
District:	Village:	Ward:	

Vaccination target areas			
Dates of vaccines	First:	Second:	
Date AEFI started:		Onset interval:	

History /Complaints:	**I**	**How many times?**		I	**Other complaints?**
Nausea Vomiting	Yes / No I			I	
Diarrhoea	Yes / No I			I	
Abdominal pain	Yes / No I			I	
Fever	Yes / No I			I	
Other:	Yes / No I				
Duration:	I				

On examination:	Temp:	BP:	PR:		RR:

Name of investigator:
Post:
Signature:
Date:

ANNEX 5: IEC Materials

हैजा रोग सम्बन्धि जानकारी लिनुहोस् !!

"हैजा" कस्तो रोग हो ?

हैजा एक छिटै सर्ने सरुवा रोग हो। यदि यसको उपचार समयमै नभएमा यसले ज्यान पनि लिन सक्छ।
हैजा ब्याक्टेरियाबाट हुने रोग हो। वान्ता हुनु, पातलो दिसा हुनु र जीउ शिथिल हुनु यस रोगको लक्षणहरु हुन्।

"हैजा" रोग कसरी सर्छ ?

हैजा रोग दुषित पानी र खानाबाट सर्दछ।

"हैजा" रोग लागेको शंका लागेमा के गर्नु पर्छ ?

हैजाका बिरामीलाई तुरुन्त नजिकैको स्वास्थ्य संस्थामा उपचारको लागि लैजानु पर्दछ। यदि उचित र समयमा उपचार नभएमा बिरामी मर्न पनि सक्छन।

हैजा रोग बाट बच्न के गर्नु पर्दछ ?

हैजाबाट बच्नको लागि स्वच्छ पानी, सफा चर्पीको प्रयोग र साबुन पानीले हात धुनँ पर्दछ।

स्वच्छ पानी सफा हातहरु सफा भाँडाकुँडाहरु

हैजा विरुद्धको खोप दिनु पर्छ। यो खोप प्रभावकारी र सुरक्षित छ।

ANNEX 6: Example Situation Report

Department of Health Services (DoHS)

Epidemiology and Disease Control Division (EDCD)

HOSPITAL-BASED SURVEILLANCE OF CHOLERA AND ACUTE GASTRO ENTERITIS CASES IN THE KATHMANDU VALLEY - DAILY SITUATION UPDATE[1]

Date: as of 28 July 2016

Key points and interventions

- 24 cholera cases were confirmed by the National Public Health Laboratory so far since 30 June, 6 cases in Kathmandu and 18 cases in Lalitpur,
- Four additional cases were diagnosed in Patan hospital,
- WASH and social mobilization activities are taking place in wards 10, 11 and 12 in Thaiba, including door-to-door visit, distribution of PIYUSH, awareness messages.

Table 1: Number of Acute Gastro Enteritis (AGE) and cholera cases reported, by hospital (excludes zero reporting)

Hospitals	Number of Acute Watery Diarrhoea		Number of probable cholera cases[2]		Number of confirmed cholera cases[3]	
	on 27/7	Cumulative (since 1/6)	on 27/7	Cumulative (since 1/6)	on 27/7	Cumulative (since 1/6)
STIDTeku, Kathmandu		224		3		3
Kanti Children Hospital, Kathmandu		35		1		1
Kathmandu Medical College, Kathmandu		77				
Nepal Medical College, Kathmandu		52				
Birendra Army Hospital, Kathmandu		33				
Central Prison Hospital, Kathmandu		2				
Patan Hospital, Lalitpur	6	141	4	22	3	18
KIST Medical College, Lalitpur		73		2		2
Bhaktapur Hospital		121				
Sidhi Memorial Hospital, Bhaktapur						
Dulikhel Hospital						
Total	**6**	**758**		**28**		**24**

1 The cases reported here include the new cases registered in the hospitals during the last 24 hours.

2 Number of cholera cases confirmed by hospitals using Dipstick Rapid Diagnostic Tests or culture confirmed (TCBS and cholera-specific biochemical confirmation).

3 Number of cholera cases confirmed by NPHL, including serology.Please note that the WHO and EWARS case definitions includes children above 5 years, but one year age cut-off is used for the purpose of this active surveillance system.

Figure 1: Number of Acute Gastro-Enteritis cases, probable and confirmed cholera cases (N=22) reported to EDCD as of 28 July 2016

Acute Watery Diarrhoea is not necessarily reported daily by all hospitals and the decreasing trend observed after 8 July can be explained by under-reporting. Cholera cases are still sporadic and are expected in this season.

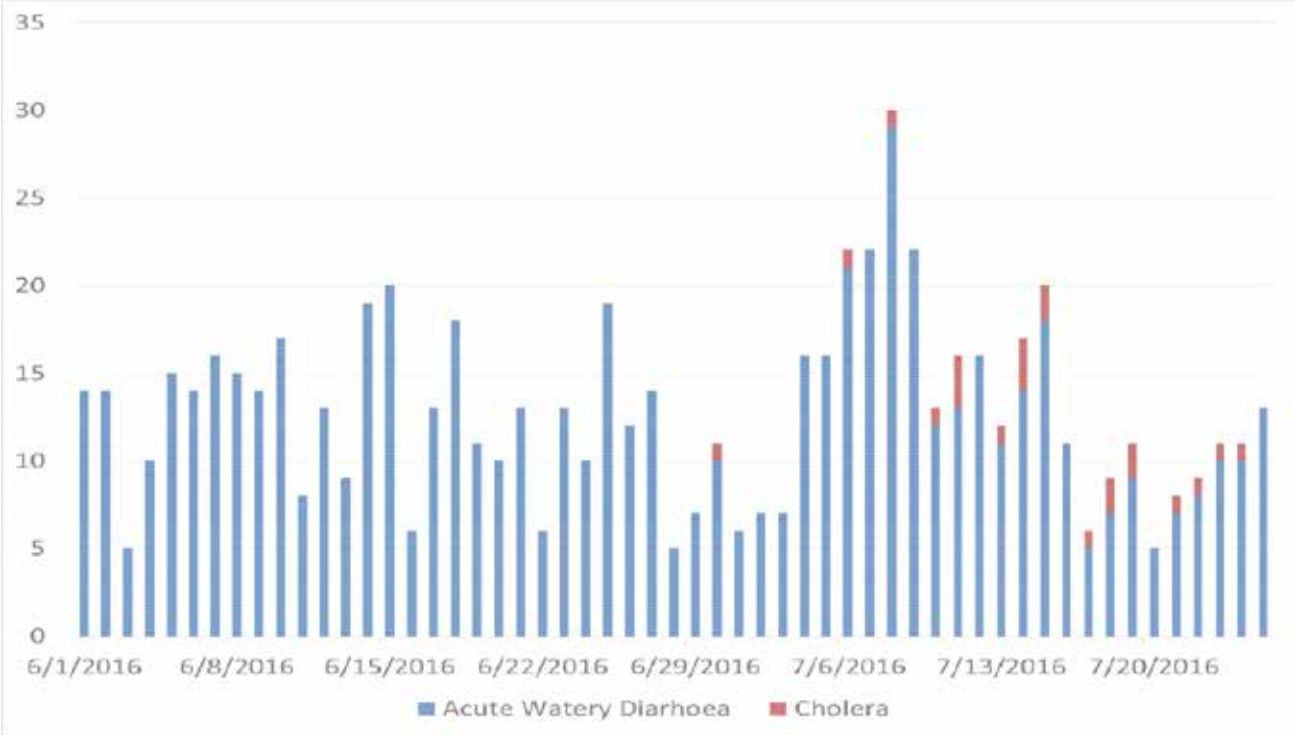


Figure 2: Distribution of cholera and acute watery diarrhoea in the Kathmandu Valley

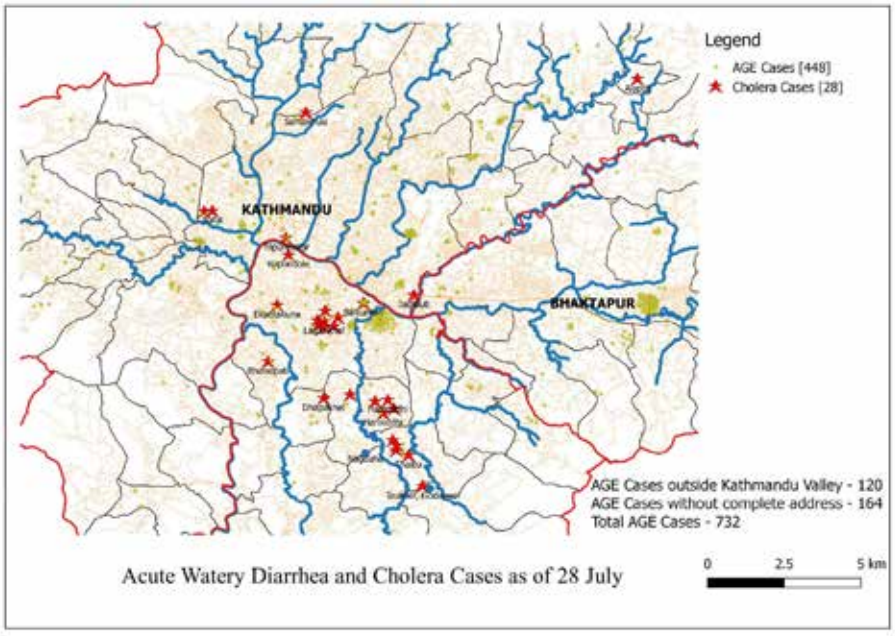


	Table 2: Number of cholera cases reported by area as of 28 July					
District	**Area**	**Week 30**	**Week 29**	**Week 28**	**Previous**	**Total**
Kathmandu	Bafal				2	2
	Tripureshor				1	1
	Samakusi			1		1
	Dacchhi/Alapot		1			1
	Jadibuti (Koteshwor)		1			1
Lalitpur	Lagankhel, Sundhara, Balkumari		2	6		8
	Baisepati			1		1
	Ekantakuna		1			1
	Kupundole		1			1
	Dhapakel		2			2
	Harisiddhi	3	1			4
	Thaiba/Godawari	1	1	2	1	5
Total		4	10	10	4	28

Acknowledgments: this bulletin is prepared by the CTI network, including expert focal points from 11 hospitals of the Kathmandu Valley reporting to EDCD and NPHL and supported by UNICEF, the World Health Organization, Johns Hopkins University, and The Group For Technical Assistance.

ANNEX 7a: Risk Factor Form for Household Investigation

Patient ID	Household ID	Patient Name	Date of Investigation	Investigation Team	District	VDC	Category	Age	Sex	Occupation	Phone

Living Since	Diarrhea Past 2 Weeks	Date Onset	Symptoms	Hospital Admitted	Drinking Water	Cleansing Water	Food Consumed Outside	Travel 1 Week	Travel Place	Primary Water Source	Secondary Water Source

Household Water Treatment	Drinking Water Storage	Storage Changed Recently	Water Quantity	Hand Washing	Water Sample Taken	Toilet	Open Defecation	Soap	Waste	Sludge

ANNEX 7b: Variable Definitions

Variable Name	Definition	Code
Sex	Male	M
	Female	F
DiarrhoeaPast2Weeks	Yes	Y
	No	N
Symptoms	Diarrhea	1
	Vomiting	2
	Abdominal Pain	3
	Fever	4
	Others	5
Hospital Admitted	Yes	Y
	No	N
Drinking Water	Tap	Tap
	Well	Well
	Bottle	Bottle
	Jar	Jar
	Tanker	Tanker
Food Consumed Outside	Yes	Y
	No	N
Travel1Week	Yes	Y
	No	N
Primary Source	Piped water; piped into dwellling; piped to yard	Piped Water
	Public tap/standpipe	Public Tap
	Tube well or borehole; dug well; protected well; unprotected well	Tube Well
	Water from spring; protected spring; unprotected spring	Spring Water
	Rain water	Rain Water
	Tanker truck	Tanker
	Surface water(river/dam); lake; pond; stream; canal	Surface Water
	Irrigation channel	Irrigation Channel
	Stone tap/dhara	Stone Tap
	Bottled water	Bottled Water
	Jar water	Jar Water
	Others	Others
Secondary Source	Piped water; piped into dwelling; piped to yard	Piped Water
	Public tap/standpipe	Public Tap
	Tube well or borehole; dug well; protected well; unprotected well	Tube Well
	Water from spring; protected spring; unprotected spring	Spring Water
	Rain water	Rain Water
	Tanker truck	Tanker
	Surface water(river/dam); lake; pond; stream; canal	Surface Water

Variable Name	Definition	Code
	Irrigation channel	Irrigation Channel
	Stone tap/dhara	Stone Tap
	Bottled water	Bottled Water
	Jar water	Jar Water
	None	None
	Others	Others
Household Water Treatment	Boiled	Boiled
	Add bleach/chorine	Chlorine
	Add piyush/water guard	Piyush/Water Guard
	Strain through a cloth	Cloth Filter
	Use water filter (ceramic/biosand/colloidal filter)	Ceramic Filter
	Solar disinfection	SoDis
	Letting stand and settle	Stand and Settle
	None	None
	Others	Others
Storage Changed Recently	Yes	Y
	No	N
Hand Washing	Yes	Y
	No	N
Toilet	Flush or pour flush toilet	Flush Toilet
	Water seal latrine	Water Seal Latrine
	Pit latrine	Pit Latrine
	Ventilated improved pit latrine	VIP Latrine
	Pit latrine with slab	Slab Pit Latrine
	Pit latrine without slab/open pit	Open Pit
	Composing toilet	Composing Toilet
	Bucket toilet	Bucket Toilet
	No facility/bush/field	None
	Others	Others
Open Defecation	Yes	Y
	No	N
Soap	Yes	Y
	No	N

ANNEX 8: M&E Indicators

Components	Indicator	Numerator	Denominator	Means of verification
Surveillance	Proportion of Steering Committee meetings held (expected monthly)	Number of meetings with minutes	Total number of meetings planned	Meeting minutes
	Routine monitoring of water quality (expected monthly)	Number of reports by DWSS to the Task Force for Cholera Control	Total number of Task Force meetings	DWSS water quality reports Meeting minutes
	Completeness of hospital reporting (including zero reporting)	Number of reports from the hospital	Expected number of hospital reports	Daily / Weekly line listings
	Timeliness of reporting cases to the EWARS system	Average time from hospital admission to EWARS reporting		Daily / Weekly line listings Hospital records
	Timely distribution of situation reports during non-outbreak (expected weekly)	Number of weekly report distributed on time	Total number of non-outbreak reporting weeks	Situation reports
	Timely distribution of situation reports during outbreak (expected daily)	Number of daily report distributed on time	Total number of outbreak reporting days	Situation reports
Response	Number of reported cholera cases that are investigated	Number of cases investigated	Number of cases reported	Situation report
	Presence of all required RRT members on investigations	Number of days with all RRT members present	Total number of days on investigation	Attendance, Outbreak Reporting form
	Number of meetings held by Task Force for Cholera Control (expected bi-monthly throughout the monsoon season)	Number of meetings with minutes	Total number of Task Force meetings planned	Meeting minutes
	Adequate availability of resources for WASH response	Number of districts reporting a shortage of WASH resources	Total number of districts	Stock Book, LMIS Report
	Adequate availability of resources for IEC/BCC response	Number of districts reporting a shortage of IEC/ BCC resources	Total number of districts	Stock Book, LMIS Report

Components	Indicator	Numerator	Denominator	Means of verification
Response	Adequate availability of vaccine	Number of cholera cases for which vaccine was not available	Total number of cholera cases	Stock Book, LMIS Report
	Completeness of risk factor reporting	Number of completed risk factor reporting forms	Total number of cholera cases	Risk Factor Forms Situation Reports
	Need for contingency stocks	Number of requests for additional supplies		NPHL Stock Book, LMIS Report
	Number of districts with established RRTs	Number of district with RRT	Total number of districts	DoHS Annual Report
Laboratory	Timeliness of reporting lab results to the EWARS system	Average time from the sample being sent to NPHL to EWARS reporting the case as confirmed		NPHL registers Situation reports
	Number of samples sent properly to NPHL (analyzable samples)	Number of sample sent properly	Total number of samples sent to NPHL	NPHL registers
	Number of samples received with culture results	Number of culture results available	Total number of samples sent to NPHL for testing	NPHL registers
	Laboratory representation in the steering committee meeting	Number of meetings with NPHL representation	Total number of Steering Committee meetings	Meeting minutes
	Adequate availability of RDTs at the hospital level	Number of hospitals requesting additional RDTs	Total number of sentinel site hospitals	NPHL Stock Book
	Adequate availability of lab supplies at the hospital level	Number of hospitals requesting additional lab supplies	Total number of sentinel site hospitals	NPHL Stock Book
	Adequate availability of lab supplies at the national lab	Number of requests from NPHL for additional supplies		NPHL Stock Book
	Routine documentation of antimicrobial resistance (testing expected for 10 cases every 2 weeks)	Number of antimicrobial disc diffusion tests performed	Expected number of tests	NPHL registers AMR report

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CURRICULUM VITAE

MELLISA ROSKOSKY, MSPH CPH CCRP

HOME
917 S Curley Street
Baltimore, MD, 21224
864.940.9247
m.roskosky@gmail.com

WORK
The Geneva Foundation
917 Pacific Ave, Tacoma, WA 98402
864.940.9247
mroskosky@genevausa.org

EDUCATION

Doctor of Philosophy (Ph.D.) December 2017
Johns Hopkins Bloomberg School of Public Health, Baltimore, MD
Department of International Health
Dissertation area: Methods in Global Cholera Surveillance and Control (Dr. David Sack)

Master of Science in Public Health May 2012
Johns Hopkins Bloomberg School of Public Health, Baltimore, MD
Department of International Health
Concentration: Global Disease Epidemiology and Control
Honors: Global Heath Field Experience Grant Recipient (2011)
Relevant Coursework: 4 terms of Epidemiologic Methods, 4 terms of Biostatistics, International Health, Genetic Epidemiology, Budgeting and Finance, Vaccine Development and Application, Vaccine Policy Issues, Data Management Methods
Certificate in Vaccine Sciences, May 2011

Bachelor of Science in Microbiology May 2010
Clemson University, Clemson, SC
Concentration: Biomedicine
Honors: Summa Cum Laude, General and Departmental Honors, Palmetto Fellows Scholar (4 year merit scholarship), Phi Kappa Phi, Trustee Scholarship (4 year merit scholarship)

PUBLIC HEALTH EXPERIENCE

Data Manager 10/2017 – Present
The Geneva Foundation, Tacoma, WA
Data manager and analyst for a phase III Department of Defense-supported clinical trial to validate a non-invasive diagnostic device for acute compartment syndrome.
• Participate study design and protocol development
• Design and test case report forms and other data collection instruments
• Design and maintain study databases in RedCap
• Perform data cleaning and analyses using R and STATA statistical software

Consultant 1/2015 – 10/2017
AOC Foundation, Athens, GA
• Protocol and grant preparation
• Data analysis and report generation
• Manuscript preparation

Research Lab Assistant 9/2014 – Present
Enterics Lab, Johns Hopkins University Bloomberg School of Public Health
Principal Investigator: Dr. David Sack
• Perform DNA extractions from environmental samples
• Sample processing for in-patient clinical trials

Clinical Research Program Manager 4/2012 – 12/2014
The Geneva Foundation, Local Site: Athens Orthopedic Clinic, Athens, GA
Data/regulatory manager for a series of medical device and clinical studies, including trials conducted as part of a Department of Defense-supported research project to validate a non-invasive diagnostic device for acute compartment syndrome.
• Performed data cleaning and analyses using STATA statistical software
• Designed and maintained study databases in Microsoft Access
• Designed Case Report Forms and other data collection instruments
• Formulated study design and procedures
• Prepared protocols and grant proposals
• Maintained study documents, including IRB and HRPO approvals
• Managed follow-up data collection and study coordinators at multiple sites
• Composed abstracts and manuscripts for publication in peer-reviewed journals

Research Assistant 6/2011 – 2/2012
Stellenbosch University Centre for Infectious Diseases, Cape Town, South Africa
HIV-Associated Nephropathy (HIVAN) study, Principal Investigator: Dr. Jean Nachega
Thesis topic: Are Gene Polymorphisms in Chromosome 22 Risk Factors for HIVAN in South African Adults: A Pilot Study.
• Supported the design and creation of an Access database for prospective and retrospective data
• Contributed to the development of the study's standard operating procedures
• Performed a comprehensive literature review of HIVAN
• Managed data collection from medical records and pathology as part of a retrospective analysis of HIVAN in South Africans

Research Assistant 11/2010 – 6/2011
Johns Hopkins Center for American Indian Health, Baltimore, MD
PCV 13 post-vaccination study, Principal Investigator: Dr. Katherine O'Brien
• Managed data collection from Navajo and Apache Native American communities in New Mexico and Arizona
• Maintained study documents
• Performed survey data entry and clean-up related to pneumonia risk factors, post vaccination safety data, and laboratory results
• Cleaned and analyzed data on *Haemophilus influenza* disease in the Navajo and Apache children using STATA statistical software

Source Community Consultant 10/2011 – Present
Back on My Feet, Baltimore, MD
• Reviewed previous grant applications and current grant writing processes to identify areas for improvement based on best practices
• Created a template for organizing metrics collected in the BoMF program

Newborn Holistic Ministries, Baltimore, MD
• Prepared a volunteer handbook and recruitment protocols
• Strategized with team on logistical aspects of volunteer positions and delegation of responsibilities

Baltimore City Health Dept. and Westside Public Outreach, Baltimore, MD
• Developed an infographic summarizing the key findings of Baltimore City's Health Impact Assessment (HIA) document to be given to stakeholders
• Identified key stakeholders within the city with which to share the results of the HIA

United Ministries, Baltimore, MD
• Developed a curriculum with a team of student consultants for an educational workshop on homelessness
• Created a homelessness fact-sheet targeted to youth and centered around advocacy for change

House of Ruth Maryland, Baltimore, MD
• Performed literature reviews on what services are desired by victims of domestic violence
• Presented results of the reviews and their implications for practice to organization leaders

Jewel House Inc., Baltimore, MD
• Cooperated with a team to develop a grant application aimed to help Jewel House provide mental health services and support to teen parents
• Researched and wrote a detailed budget scheme for the Psychiatric Rehabilitation Program

Project PLASE, Baltimore, MD
• Assisted in the development of a Pepsi Refresh grant application for $25,000 to work on food programs for Baltimore's Homeless
• Prepared a fund-raising scheme: "3$ for 3 Hot Meals/Day"

ICHEC India Housing Project Volunteer Summer 2009
Brussels, Belgium and Tamil Nadu, India
• Led and developed a fund-raising scheme for program/building supplies as well as transportation and living expenses
• Assisted in the building of houses in an Indian village
• Taught English at a local school
• Collaborated with local village doctors on teaching good health practices within cultural boundaries and norms
• Adapted to very basic living conditions in a rural community with non-English speaking students

OTHER RESEARCH EXPERIENCE
Research/ Lab Assistant 8/2009 – 5/2010
Clemson University, Clemson, SC
• Developed a research plan on how to assess efficacy of protein treatment on cancer cells
• Conducted bench research pertaining to potential breast cancer treatment, including intensive cell culture work
• Generated an honors thesis entitled, Investigation of the Effects of G3 Protein Treatment on Breast Cancer Cells

Creative Inquiry Team Member 1/2009 – 5/2010
Clemson University, Clemson, SC
• Created a research protocol for the study of bacterial colonization of automatic hand-dryers
• Collected samples and organized data relevant to the hypothesis
• Presented findings at a Clemson University Undergraduate Research Colloquium

TEACHING EXPERIENCE
Teaching Assistant 10/2011 – Present
Department of International Health, JHSPH
Clinical Vaccine Trials and Good Clinical Practice
• Develop course materials and lectures with instructor, maintain course website, grade assignments, communicate with students, host online live-talk sessions with students; 3 Terms (Karen Charron)

Global Disease Control Programs and Policies

• Grade assignments, communicate with students; 6 Terms (Dr. Alain Labrique & Dr. Summer Rosenstock)

PEER-REVIEWED PUBLICATIONS

Dixon K, Broussard A, **Roskosky M**, Shuler M. *SpO2 and Pulse Rate Data: A Comparison of Current Technologies during Sustained Shivering in Post-Operative Patients.* Journal of Anesthesia and Clinical Research. 2017. 8:4.

Reisman W, Shuler M, **Roskosky M**, Kinsey T, Freedman B. *Use of Near Infrared Spectroscopy to Detect Sustained Hyperaemia Following of Lower Extremity Trauma.* Military Medicine. 2016. 2:111.

Couch L, **Roskosky M**, Shuler M, Freedman B. *Correlation between skin pigment and NIRS values: a comparison of three commercially available devices*. American Journal of Analytical Chemistry. 2015. 6:911-16.

Johnson A, **Roskosky M**, Shuler M, Freedman B. *Depth Penetration of Near Infrared Spectroscopy in the Obese*. Journal of Trauma and Treatment. 2015. 4:263.

Kovalenko B, **Roskosky M**, Shuler M, Freedman B. *Effects of Ambient Light on Near Infrared Spectroscopy.* Journal of Trauma and Treatment. 2015. 4:258.

Austin A, Green S, Ahsan S, **Roskosky M**, Shuler M. *Cadaveric Study for Appropriate Screw Length for Distal Radius Stabilization Using Volar Plate Fixation.* The American Journal of Orthopedics. 2015. 44(8):369-72.

Roskosky M, Robinson G, Shuler M, Freedman B. *Subcutaneous Depth in a Traumatized Lower Extremity.* Journal of Trauma and Acute Care Surgery. Vol 77, 3. Supplement 2. 2014.

Cole A, **Roskosky M**, Shuler M, Freedman B. *Near infrared spectroscopy and lower extremity acute compartment syndrome: a review of the literature.* Journal of Trauma and Treatment. 2014. S2:003.

BOOK CHAPTERS

Shuler MS, **M Roskosky**, Z Elstad, J Kinney. "Fractures of the Phalanx." Principles of Hand Surgery and Therapy. 3rd ed. Ed. Thomas Trumble, Ghazi Rayan, Mark Baratz. Philidelphia: Saunders. (2016)

Shuler MS, **M Roskosky**, BA Freedman. "Compartment Syndromes." Skeletal Trauma: Basic Science, Management, and Reconstruction. 5th ed. Ed. Bruce Browner, MD, Alan Levine, MD, Jesse Jupiter, MD, Peter Trafton, MD, Christian Krettek, MD. Philidelphia: Saunders. (2014)

ABSTRACTS AND PRESENTATIONS

Roskosky M, Ali M, Acharya J, Li S, Stine O, Sack D. *Minimal genetic diversity and spatial clustering of cholera cases in the Kathmandu Valley: Implications for a ring-vaccination strategy.* International Conference on Emerging Infectious Diseases, February 2018. (Podium)

Roskosky M, Ali M, Upreti SR, Sack D. *Spatial Clustering of Cholera Cases in the Kathmandu Valley: Implications for a Ring-Vaccination Strategy.* JHSPH GIS Day, November 2017. (Poster)

Roskosky M, Acharya B, Shakya G, Karki K, Bajracharya D, Sack D. *Feasibility of a Comprehensive Targeted Cholera Intervention in Kathmandu Valley, Nepal.* American Society for Tropical Medicine and Hygiene, November 2017. (Poster)

Roskosky M, Acharya B, Shakya G, Karki K, Bajracharya D, Sack D. *Feasibility of a Comprehensive Targeted Cholera Intervention in Kathmandu Valley, Nepal.* JHSPH Vaccine Day, April 2017. (Poster)

Roskosky M, Acharya B, Shakya G, Karki K, Bajracharya D, Sack D. *Feasibility of a Comprehensive Targeted Cholera Intervention in Kathmandu Valley, Nepal.* International Conference on Emerging Infectious Diseases, February 2017. (Poster)

Roskosky M, Kinsey T, Shuler M, Reisman W, Ogburn C, Freedman B. *Continual Near Infrared Spectroscopy Monitoring in the Injured Extremity at Risk for Acute Compartment Syndrome—Results of a Prospective, FDA-IDE Trial.* American Academy of Orthopedic Surgeons, March 2017.(Podium)

Roskosky M, Kinsey T, Shuler M, Reisman W, Ogburn C, Freedman B. *Continual Near Infrared Spectroscopy Monitoring in the Injured Extremity at Risk for Acute Compartment Syndrome—Results of a Prospective, FDA-IDE Trial.* Society of Military Orthopedic Surgeons, October 2016. (Podium)

- SOMOS Founder's Paper Award

Roskosky M, Kinsey T, Shuler M, Reisman W, Ogburn C, Freedman B. *Continual Near Infrared Spectroscopy Monitoring in Acute Compartment Syndrome: Lessons Learned from a Decade of War.* Military Health System Research Symposium, August 2015. (Poster)

Budsberg S, Shuler M, **Roskosky M**, Uhl E, Hansen M, Freedman B. Correlation of NIRS and histological muscle damage in a prolonged trauma/infusion model of extremity compartment syndrome (ECS) – assessing NIRS ability to detect the clinical consequence of delayed ECS. Orthopedic Research Society Annual Meeting, March 2015. (Poster)

Reisman W, Cole A, **Roskosky M**, Shuler M, Andras L, Moore T. *Near-Infrared Spectroscopy in the Sub-Acute Setting of Lower Extremity Trauma*. 2014 Military Health Systems Research Symposium, August 2014. (Podium)

Roskosky M, Cole A, Epstein E, Shuler M. *Suture Fixation Versus Reconstruction in CMC Arthroplasty: Double-blind RCT.* American Orthopedic Association / Canadian Orthopedic Association Joint Meeting, June 2014. (Podium)

Roskosky M, Cole A, Epstein E, Shuler M. *Suture Fixation Versus Reconstruction in CMC Arthroplasty: Double-blind RCT.* American Academy of Orthopedic Surgeons, March 2014. (Podium)

Roskosky M, Robinson G, Shuler M, Freedman B. *Subcutaneous Depth in a Traumatized Lower Extremity*. Society of Military Orthopedic Surgeons 55th Annual Meeting, December 2013. (Podium)

Roskosky M, Shuler M. *Suture Fixation Versus Reconstruction in CMC Arthroplasty: Double-blind RCT.* 68th Annual Meeting of the American Society for Surgery of the Hand, October 2013. (Podium)

Couch L, **Roskosky M**, Shuler M, Freedman B. *Correlation between skin pigment and NIRS values: a comparison of three commercially available devices.* Medical College of Georgia: 2013 Med Scholars Program, September 2013. (Poster)

Roskosky M, Robinson G, Shuler M, Freedman B. *Subcutaneous Depth in a Traumatized Lower Extremity.* Military Health System Research Symposium, August 2013. (Podium)

Roskosky M, Robinson G, Shuler M, Freedman B. *Subcutaneous Depth in a Traumatized Lower Extremity.* Southern Orthopedic Association 30th Annual Meeting, July 2013. (Poster)

Johnson A, **Roskosky M**, Shuler M, Freedman B. *Depth Penetration of Near Infrared Spectroscopy in the Obese*. Medical College of Georgia: 2012 Med Scholars Program, September 2012. (Poster)
Roskosky, M. *HIV-Associated Nephropathy in South African Adults: A Retrospective Analysis.* JHSPH Global Health Day, March 2012. (Poster)

WORKS IN PROGRESS

Peer-Reviewed Publications

Shuler M, **Roskosky M**, Kinsey T, Glaser D and Freedman B. *Continual Near Infrared Spectroscopy Monitoring in the Injured Extremity at Risk for Acute Compartment Syndrome—Results of a Prospective, FDA-IDE Trial.* Bone and Joint Journal. (Submitted)

Sekine K, **Roskosky, M** and Maskey A. *Lessons learned from enhancing sentinel surveillance for cholera in post-earthquake Nepal in 2016*. The American Journal of Tropical Medicine and Hygiene. (Submitted)

Sekine K, **Roskosky, M** and Maskey A. *Lessons learned from water, sanitation and hygiene for cholera control in post-earthquake Nepal in 2016*. WHO Bulletin. (Submitted)

Wanderman N, Mitchell S, Yuan B, Reisman W, **Roskosky M**, Shuler M, Freedman B. The Use of Near Infrared Spectroscopy in the Diagnosis of Acute Compartment Syndrome. Trials. (To be submitted)

Quinet M, Dixon K, Savitz J, **Roskosky M**, Shuler M. *Effectiveness of Amion Patch Tissue Grafts as Nerve Wraps Revision Carpal/Cubital Tunnel Release in Recurrent Carpal/Cubital Tunnel Syndrome*. The American Journal of Orthopedics. (To be submitted)

CURRENT STUDIES

2017 – 2021. *NIRS to reduce the prophylactic fasciotomies for and missed cases of acute compartment syndrome in soilders injured in OEF/OIF: Interventional study*. Atlanta, Georgia and Rochester, MN. A multi-site abbreviated IDE trial supported by the Department of Defense Congressionally Directed Joint Warfighter Medical Research Program, Award. number: W81XWH-17-C-0029 (Active)

2012 – 2018. *Delivering Oral Cholera Vaccine Effectively (DOVE),* Baltimore, Maryland. Supported by the Bill and Melinda Gates Foundation, Award number: 113844. (Active)

2012 – 2017. *The use of near infrared spectroscopy in the diagnosis of acute compartment syndrome*, Atlanta, Georgia. A multi-site abbreviated IDE trial supported by the Department of Defense Congressionally Directed Medical Research Program, Award number: DR080018. (Active)

SKILLS AND CERTIFICATIONS

Certified in Public Health (2015 – present)
Certified Clinical Research Professional – Society of Clinical Research Associates (2013 – present)
CITI, Human Research Curriculum (2010 – present)
Clinical Vaccine Trials and Good Clinical Practice (2011)
Vaccine Science (2011)
Adult and Pediatric First Aid / CPR / AED – American Red Cross (Current)
Trained in statistical programming and data manipulation in STATA, R, ArcGIS, and Microsoft Excel
Experienced in database design using Microsoft Access and RedCap
Competent in Endnote and Refworks

COMMUNITY INVOLVEMENT

Counselor, Camp Happy Days (2008 – present)
- Five Years of Service Award
- Eight Years of Service Award

Judge/Reader, Georgia Junior Science and Humanities Symposium (2013 – 2018)
Junior Judge, Georgia Science and Engineering Fair (2012 – 2014)

PROFESSIONAL AFFILIATIONS

Global Health Council (2010 – present)
Society of Clinical Research Associates (2013 – present)
Fund-raising Coordinator for the JHSPH African Public Health Network (2010 – 2012)